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# THE RELATIONSHIP OF GROWTH HORMONES AND FRUIT DEVELOPMENT

By Robert M. Muir*

Department of Botany, University of Michigan†

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Fruit development is normally the result of pollination and fertilization although numerous instances of parthenocarpy and parthenogenesis have been reported in the literature. It has been shown (Muir[11]) that considerable quantities of diffusible growth hormones are released in the style and ovary of *Nicotiana tabacum* following pollination and fertilization. The investigations of Fitting,[5] Laibach,[10] Thimann[14] and others have indicated that pollen is a relatively rich source of growth hormones. With this in mind Gustafson[7] postulated that the growth hormones from the pollen grains and pollen tubes initiate the early stages of fruit development and after fertilization the developing embryo provides additional growth hormones to other portions of the ovary for its development. Van Overbeek, Conklin and Blakeslee[17] have stated that the pollen of an ordinary pollination does not contain sufficient auxin to be the sole cause of fruit development. They suggest that the substance from the pollen which initiates enlargement of the ovary and ovule may be a prosthetic group which properly combined in the ovary forms an enzyme which activates the auxin precursor. The verification of either hypothesis requires the examination of the possible sources of the growth hormones involved in fruit development on a quantitative basis.

Experiments were performed to determine (1) the amount of growth hormones in the pollen grain which could be transported through the pollen tube to the ovary; (2) the production of growth hormones during the development of the pollen tube; (3) the amount of growth hormones in the ovary before fertilization; (4) the production of growth hormones in ovary tissues by the action of pollen extracts.

*Bioassay of Growth Hormones.*—Determinations of growth hormone concentrations were made according to the modification suggested by Skoog[12] of the standard *Avena* test as described by Went and Thimann.[18]

Curvatures are expressed as the arithmetical mean of the test row of plants with the standard error of the mean and are recorded for the same agar dilution in each instance. Direct comparisons of curvatures are valid in any given experiment. To correct for differences in the sensitivity of the test plants in different experiments the curvatures are translated into the number of micrograms of indoleacetic acid required to produce an equivalent amount of curvature of the *Avena* coleoptile as is produced by the growth hormones obtained from the test material. The calculation employs the following formula:

$$\frac{\text{Micrograms Indoleacetic Acid}}{\text{Milligram of Material}} = \frac{V_1 \times C_1 \times 2.5 \times 10^{-4}}{V_2 \times C_2 \times W}$$

$V_1$ = volume of agar dilution of the extract
$V_2$ = volume of agar block applied to each coleoptile = 0.0125 ml.
$C_1$ = average curvature of coleoptile induced by the extract
$C_2$ = average curvature of coleoptile induced by $2.5 \times 10^{-4}$ micrograms of indoleacetic acid
$W$ = fresh weight of material extracted in milligrams

*Growth Hormones in Pollen Grains and Pollen Tubes.*—A weighed quantity of viable pollen was ground with powdered glass and a few drops of a 10-ml. volume of 0.1 *N* HCl until examination under the microscope revealed no whole grains. The remaining acid was used to transfer the mixture quantitatively to a separatory funnel. The pH of the mixture was adjusted to 3.0–4.0 (glass electrode) before extraction with three separate 50-ml. portions of recently distilled chloroform. The chloroform was withdrawn and evaporated until only a few ml. remained which were transferred to a small vial and taken up in agar.

Excellent pollen grain germination and pollen tube growth for the various species tested occurred on a medium composed of 1% agar and 10% sucrose. The pollen was distributed uniformly over 2 ml. of sterile medium in a sterile petri dish. A piece of moist filter paper was inserted inside the cover and the dish was placed in a darkroom at 25°C. All cultures were examined microscopically before being ground with glass, acidified and extracted. Occasionally the cultures older than 24 hrs. were found to be contaminated and were discarded.

Representative experiments for each type of pollen are presented in table 1. *Nicotiana* pollen has a relatively small amount of free hormone and no marked increase in amount occurs upon germination. The pollen of *Datura* contains twice as much free hormone as the pollen of *Nicotiana* but no increase in amount occurs following germination. Upon germination of the *Antirrhinum* pollen the growth hormone concentration increased to an amount which was three or four times that present in the

TABLE 1

CONCENTRATIONS OF GROWTH HORMONES OBTAINED BY EXTRACTION OF POLLEN GRAINS AND POLLEN TUBES

| SOURCE OF POLLEN | STAGE OF MATERIAL EXTRACTED | MG. OF POLLEN GRAINS | AVERAGE AVENA TEST CURVATURE | MICROGRAMS OF INDOLEACETIC ACID $\times 10^{-4}$ PER MG. OF POLLEN |
|---|---|---|---|---|
| *Nicotiana tabacum* | Grains | 29.0 | 4.2 ± 1.2 | 0.7 |
| | Grains | 29.3 | 2.0 ± 0.8 | 0.3 |
| | Tubes (15 Hrs.) | 14.0 | 0.0 | 0.0 |
| | Tubes (15 Hrs.) | 30.0 | 0.0 | 0.0 |
| | Tubes (25 Hrs.) | 34.5 | 0.0 | 0.0 |
| | Tubes (25 Hrs.) | 35.0 | 8.6 ± 0.8 | 1.2 |
| | Tubes (37 Hrs.) | 29.2 | 2.0 ± 0.8 | 0.3 |
| | Tubes (37 Hrs.) | 28.7 | 5.0 ± 1.1 | 0.9 |
| *Antirrhinum majus* | Grains | 30.0 | 4.7 ± 0.9 | 1.1 |
| | Tubes ( 8 Hrs.) | 30.0 | 16.7 ± 1.2 | 3.9 |
| | Tubes ( 8 Hrs.) | 30.0 | 17.3 ± 1.8 | 4.0 |
| | Tubes (14 Hrs.) | 30.0 | 17.1 ± 1.8 | 4.0 |
| | Tubes (14 Hrs.) | 30.0 | 17.4 ± 1.4 | 4.0 |
| | Tubes (14 Hrs.) | 30.0 | 14.5 ± 0.7 | 3.4 |
| | Tubes (25 Hrs.) | 30.0 | 14.9 ± 1.5 | 3.5 |
| | Tubes (25 Hrs.) | 30.0 | 13.0 ± 1.1 | 3.0 |
| *Cyclamen persicum* | Grains | 11.0 | 3.9 ± 1.5 | 2.4 |
| | Tubes ( 6 Hrs.) | 12.0 | 3.4 ± 1.1 | 1.9 |
| | Tubes ( 6 Hrs.) | 13.0 | 7.9 ± 0.8 | 4.4 |
| | Tubes (10 Hrs.) | 12.0 | 4.7 ± 0.4 | 2.6 |
| | Tubes (22 Hrs.) | 12.0 | 7.2 ± 0.7 | 4.0 |
| | Tubes (22 Hrs.) | 12.0 | 9.9 ± 0.7 | 6.0 |
| *Datura suaveolens* | Grains | 53.0 | 11.3 ± 1.1 | 1.4 |
| | Tubes ( 9 Hrs.) | 70.0 | 16.5 ± 2.5 | 1.6 |
| | Tubes ( 9 Hrs.) | 63.0 | 14.3 ± 1.6 | 1.5 |
| | Tubes (26 Hrs.) | 62.0 | 16.4 ± 1.5 | 1.8 |

grain. The pollen grains of *Cyclamen* were the richest source of hormones. The concentration of free hormone in the germinated pollen was double or triple the concentration before germination. The considerable fluctuation in the concentration of free hormone was found to be characteristic for the pollen tube growth of both *Nicotiana* and *Cyclamen*.

*Growth Hormones in Pollen Grains Subjected to Hydrolysis.*—The increased concentrations of growth hormones in the germinated pollen of *Antirrhinum* and *Cyclamen* suggests the liberation of active hormones from inactive combinations during the growth of the pollen tube. The investigations of Skoog and Thimann,[13] Wildman and Gordon,[19] Gordon[6] and Avery, Berger and White[1] all indicate the existence of growth substances both as free, active entities and in bound, inactive combinations. To release the active hormones the pollen grains were subjected to acid and alkaline hydrolysis following the methods of Avery, Berger and White.[1] Thirty mg. of pollen grains were ground with powdered glass and trans-

ferred to a large test tube with 5 ml. 1.0 *N* NaOH or 10 ml. 0.1 *N* HCl. The mixtures were autoclaved 30 min. at 15-lb. pressure (120°C.) and allowed to cool. The pH was adjusted to 3.0–4.0 with 1.0 *N* HCl and the aqueous solutions were extracted with chloroform. The concentrations of free hormones following hydrolysis of the pollen materials are presented in table 2. A remarkable uniformity of yield following hydrolysis with

TABLE 2

CONCENTRATIONS OF GROWTH HORMONES OBTAINED BY EXTRACTION OF POLLEN GRAINS FOLLOWING HYDROLYSIS

| SOURCE OF POLLEN | EXP. NO. | TYPE OF HYDROLYSIS | AVERAGE AVENA TEST CURVATURE | MICROGRAMS OF INDOLEACETIC ACID $\times 10^{-4}$ PER MG. OF POLLEN |
|---|---|---|---|---|
| *Nicotiana tabacum* | 1 | None | 0.0 | 0.0 |
| | | None | 0.0 | 0.0 |
| | | None | 0.0 | 0.0 |
| | | Alkali | 30.5 ± 1.8 | 3.6 |
| | | Alkali | 27.8 ± 1.5 | 3.3 |
| | | Alkali | 30.4 ± 3.1 | 3.6 |
| | | Acid | 0.0 | 0.0 |
| | | Acid | 0.0 | 0.0 |
| | | Acid | 0.0 | 0.0 |
| *Antirrhinum majus* | 1 | None | 4.0 ± 0.7 | 0.6 |
| | | None | 0.0 | 0.0 |
| | | Alkali | 31.6 ± 1.3 | 4.6 |
| | | Alkali | 32.0 ± 0.9 | 4.7 |
| | 2 | Alkali | 19.7 ± 1.0 | 3.0 |
| | | Acid | 20.2 ± 1.3 | 3.1 |
| | | Acid | 19.2 ± 1.0 | 3.0 |
| *Datura suaveolens* | 1 | None | 22.4 ± 1.3 | 3.3 |
| | | Alkali | 22.6 ± 1.4 | 3.3 |
| | | Alkali | 24.4 ± 2.1 | 3.6 |
| | | Alkali | 22.7 ± 1.5 | 3.3 |
| | 2 | Alkali | 28.4 ± 1.9 | 4.3 |
| | | Alkali | 27.6 ± 1.8 | 4.2 |
| | | Acid | 0.0 | 0.0 |
| | | Acid | 0.0 | 0.0 |

alkali is demonstrated. These yields are the same as those obtained following germination of *Antirrhinum* and *Cyclamen* pollen but are much larger than the yields obtained from germinated pollen of *Nicotiana*. Although larger amounts of hormones were obtained in these experiments with pollen of *Datura* collected in June than were obtained in the germination tests with pollen collected in April, the hydrolysis of the material did not increase the yield of free hormone, which fact is in agreement with the demonstration that no marked change in concentration of growth substances occurred following germination of the pollen. Acid hydrolysis did not increase the yield of free hormone from *Nicotiana* pollen and decreased the yield from *Datura* pollen. Acid hydrolysis of the pollen of

*Antirrhinum* increased the yield of hormone as much as hydrolysis with alkali increased it. These data indicate that the hormones of the *Nicotiana* pollen are in a bound, inactive state and are only partially liberated during the germination of the grain. The hormones of *Antirrhinum* pollen are in a bound, inactive state for the most part but are liberated during germination. The hormones of *Datura* pollen are all present in the free, active form.

*Growth Hormones in Ovary Tissue.*—The hypothesis of the enzymatic activation of the auxin precursor in the ovary as a result of pollination made the assumption that a similar condition of active and inactive forms of the growth hormones occurred in the ovary as had been demonstrated by van Overbeek[16] for the coleoptile tip of maize seedlings. Extractions of dried ovary tissue of *Nicotiana* and *Antirrhinum* were made to investigate the occurrence of both free and bound forms of the hormones in the unpollinated pistil. The ovary tissue was dried *in vacuo* at 60°C. and ground to pass through an 80-mesh screen. Determinations were carried out with 20-mg. samples of this material.

The conversion of the bound form of the hormone to the free form by enzymatic digestion was investigated by dispersing the tissue in 10 ml. of $KH_2PO_4$-NaOH buffer solution of pH 8.0 with 3 mg. of a commercial pancreatic preparation (Fairchild). Numerous tests of this preparation have shown it to have no growth effects in the *Avena* test. Controls were prepared in which the enzyme preparation was omitted. The development of microörganisms in the digestions was prevented by adding 15 drops of toluol every 24 hrs. and tightly stoppering the flasks. Following incubation at 37°C. for 48 hrs., the pH of the mixtures was adjusted to 3.0–4.0 and the hormones were extracted from the mixtures with purified ether. The conversion of the bound form to the free hormone by hydrolysis with 1.0 *N* NaOH and 0.1 *N* HCl was determined as in the experiments with the pollen materials. The concentration of free hormone was determined by an ether extraction of the ovary tissue dispersed in acidified water for a period of 8 hrs. at 4°C.

The results of representative experiments with each type of tissue are presented in table 3. They show that the unpollinated pistil of *Nicotiana* contains no detectable free hormone but that a considerable quantity of bound hormone is present which can be converted to the active form merely by incubation in a medium of pH 8.0 and to a lesser degree by acid and alkali hydrolysis. The unpollinated pistil of *Antirrhinum* contains a small amount of free hormone but a much larger amount of bound hormone which can be converted to the active form by incubation in a medium of pH 8.0 and by acid hydrolysis but not appreciably by alkali hydrolysis.

*The Conversion of Bound Hormone to Free Hormone by Pollen Extracts.*—The demonstration of considerable quantities of bound hormone in the

TABLE 3

CONCENTRATIONS OF GROWTH HORMONES OBTAINED BY EXTRACTION OF OVARY TISSUE

| SOURCE OF TISSUE | TREATMENT | AVERAGE AVENA TEST CURVATURE | MICROGRAMS OF INDOLEACETIC ACID $\times 10^{-4}$ PER MG. OF TISSUE (FRESH WT.) |
|---|---|---|---|
| *Nicotiana tabacum* | Tryptic digestion | 37.3 ± 2.5 | 1.5 |
| | Tryptic digestion | 32.0 ± 1.9 | 1.3 |
| | Control incubation | 38.0 ± 1.5 | 1.5 |
| | Acid hydrolysis | 10.0 ± 0.2 | 0.4 |
| | Acid hydrolysis | 10.2 ± 0.7 | 0.4 |
| | Alkali hydrolysis | 12.4 ± 0.8 | 0.5 |
| | Alkali hydrolysis | 9.1 ± 1.0 | 0.4 |
| | None | 0.0 | 0.0 |
| | None | 0.0 | 0.0 |
| *Antirrhinum majus* | Tryptic digestion | 26.2 ± 2.0 | 1.3 |
| | Control incubation | 19.5 ± 1.9 | 1.0 |
| | Control incubation | 14.3 ± 1.0 | 0.7 |
| | Acid hydrolysis | 14.0 ± 0.8 | 0.7 |
| | Alkali hydrolysis | 2.3 ± 0.4 | 0.1 |
| | Alkali hydrolysis | 3.9 ± 0.7 | 0.2 |
| | None | 5.4 ± 0.5 | 0.3 |
| | None | 3.1 ± 0.8 | 0.2 |

TABLE 4

THE CONVERSION OF BOUND HORMONE TO FREE HORMONE IN OVARY TISSUE OF NICOTIANA BY POLLEN EXTRACTS

| EXP. NO. | MEDIUM | PREPARATION | AVERAGE AVENA TEST CURVATURE |
|---|---|---|---|
| 1 | Distilled water, pH 6.3 | Ovary tissue + pollen extract | 22.8 ± 1.2 |
| | | Ovary tissue | 0.0 |
| | | Ovary tissue | 0.0 |
| | | Pollen extract | 0.0 |
| | | Pollen extract | 0.0 |
| | Buffer solution, pH 5.9 | Ovary tissue + pollen extract | 27.0 ± 2.6 |
| | | Ovary tissue + pollen extract | 20.0 ± 1.1 |
| | | Ovary tissue | 0.0 |
| | | Ovary tissue | 0.0 |
| | | Pollen extract | 0.0 |
| | | Pollen extract | 0.0 |
| 2 | Buffer solution, pH 5.9 | Ovary tissue + pollen extract | 15.0 ± 1.3 |
| | | Ovary tissue + pollen extract | 14.7 ± 1.4 |
| | | Ovary tissue | 5.5 ± 1.2 |
| | | Ovary tissue | 4.5 ± 1.1 |
| | | Pollen extract | 0.0 |
| | | Pollen extract | 0.0 |
| | Buffer solution, pH 8.0 | Ovary tissue + pollen extract | 22.7 ± 1.6 |
| | | Ovary tissue + pollen extract | 40.2 ± 3.2 |
| | | Ovary tissue | 34.1 ± 1.7 |
| | | Ovary tissue | 34.6 ± 1.7 |
| | | Pollen extract | 0.0 |

ovary of *Nicotiana* and *Antirrhinum* suggested the investigation of substances in pollen which might bring about the activation of the hormones as hypothesized by van Overbeek, Conklin and Blakeslee. Extracts were made by grinding 0.1 gm. of *Nicotiana* pollen grains with glass dispersing the material in distilled water and toluol, and agitating the mixture mechanically for 37 hrs. at 13°C. The suspension was centrifuged to remove the glass and pollen debris and 16 ml. of a yellowish, slightly turbid extract were obtained. Twenty mg. of dried ovary tissue of *Nicotiana* (unpollinated pistils) were dispersed in 10-ml. portions of distilled water and $KH_2PO_4$-NaOH buffer solutions of pH 8.0 and 5.9. Two ml. of the pollen extract were added to one set of dispersions, 2 ml. of distilled water were added to a control set and 2 ml. of the pollen extract were added to 10 ml. of water and buffer solutions which did not contain ovary tissue. All mixtures were incubated at 37°C. for 24 hrs. after 20 drops of toluol had been added and the flasks tightly stoppered. After incubation the pH of the mixtures was adjusted to 3.0–4.0 and extractions were made with ether.

The results of two experiments presented in table 4 show that in distilled water and buffer solution of pH 5.9 the mixture of ovary tissue and pollen extract yielded large quantities of free hormones whereas the ovary tissue alone and the pollen extract alone yielded none or little free hormone. In buffer solution of pH 8.0 the same yields of free hormones were obtained from the ovary tissue alone as were obtained from the mixture of ovary tissue and pollen extract. The conversion of the bound form of the hormone to the free form in an alkaline medium has been observed previously (see table 3). These experiments demonstrate that the extract of the pollen contains a substance or substances which can convert the bound hormones in the dried ovary tissue to free hormones.

*Discussion and Interpretation of Experimental Results.*—Since the pollen grains, pollen tubes and ovaries have all been shown to contain growth substances, the source of the diffusible hormones detected in the ovary following pollination and fertilization can be established only by a comparison of the amounts in the pollen and ovary tissue. Buchholz[4] has stated that 600–900 pollen grains may be regarded as a normal abundant pollination in *Datura* and van Overbeek, Conklin and Blakeslee cite this figure to support their view that the pollen has insufficient hormones to cause fruit development. Thus 1000 pollen grains would be a liberal figure for pollination. Determinations of hormone concentrations were made on a weight basis and the calculation of amounts of hormones involved in a normal pollination requires estimates of the number of grains per unit weight of pollen. Heyl[8] while studying the chemical composition of pollen estimated that there are 610 millions of grains in one gram of ragweed pollen and Ulrich[15] estimated 173 millions of grains per gram of

ragweed pollen. In this study estimates of the number of grains per gram of pollen were made by dispersing a weighed amount of pollen in a definite volume of 50% glycerol and water after wetting the grains with a drop of chloroform. Duplicate aliquots of the suspension were placed on a glass slide under a cover glass and counted with the aid of a mechanical stage. The pollen grain of *Nicotiana* has an average volume of 14,500 cubic microns and there are approximately 60 millions of grains per gram of fresh pollen. The pollen grain of *Antirrhinum* has an average volume of 5300 cubic microns and there are approximately 170 millions of grains per gram of fresh pollen.

Small yields of growth hormones were obtained from *Nicotiana* pollen grains and tubes but approximately $4.0 \times 10^{-4}$ microgram of indoleacetic acid per milligram of pollen was obtained following alkali hydrolysis and as this concentration corresponds well with maximum yields from other types of pollen it will be acceptable as the maximum concentration in *Nicotiana* pollen. Calculation reveals that the maximum concentration of growth hormones in the pollen of a normal pollination is then $6 \times 10^{-6}$ microgram of indoleacetic acid. In experiments with *Nicotiana tabacum* reported elsewhere (Muir[11]) it was found that immediately following fertilization there appear sufficient diffusible growth hormones in the ovary to produce curvatures of 30 degrees in the *Avena* test, a concentration of approximately $6 \times 10^{-4}$ microgram of indoleacetic acid, 100 times as much hormone as is contained in the pollen of a normal pollination. Similarly, following pollination there appear sufficient diffusible growth hormones in apical and basal portions of the style to produce curvatures of 10 degrees, a concentration of approximately $2 \times 10^{-4}$ microgram of indoleacetic acid, 30 times as much growth hormones as are contained in the pollen. Maximum yields of hormones from the ovary tissue of unpollinated *Nicotiana* pistils were $1.5 \times 10^{-4}$ microgram of indoleacetic acid per milligram of fresh tissue. The ovary with an average fresh weight of 34 mg. thus contained approximately $5 \times 10^{-3}$ microgram of indoleacetic acid, 800 times as much hormone as was found in the pollen. Yields of hormones obtained by extraction of unpollinated pistils were 8 times as great as the yields obtained by diffusion of fertilized ovaries.

Calculation of the amount of growth hormones in the pollen of a normal pollination for *Antirrhinum* gives a value of approximately $2 \times 10^{-6}$ microgram of indoleacetic acid. The maximum yield of hormones from the ovary tissue of unpollinated *Antirrhinum* pistils was $1.3 \times 10^{-4}$ microgram of indoleacetic acid per milligram of tissue. The ovary with an average fresh weight of 8 mg. thus contained approximately $1 \times 10^{-3}$ microgram of indoleacetic acid, 500 times as much hormone as contained in the pollen.

The above comparisons of the amounts of growth hormones in the

pollen and ovary tissue and the demonstration that incubation of water extracts of pollen with ovary tissue yields large amounts of free growth hormones establish the fact that changes in concentration of active hormones in the pistil associated with pollination and fertilization are the result of a substance or substances, other than growth hormones, brought into the pistil by the pollen tubes.

The identity of the effective substance in pollen will be indicated more definitely when the mechanism of free auxin formation in the ovary following fertilization has been established. The investigations of Skoog and Thimann[13] and Wildman and Gordon[19] suggest that in some tissues the inactive growth hormones are bound to proteins and thus the effective substance in pollen may be part of an enzyme system which brings about the release of active hormone from protein combinations. Recently Bonner and Wildman[3] have suggested that the hormone protein complex in the leaves of spinach is an active entity with the enzymatic properties of a phosphatase and that the hormone, indoleacetic acid, is formed from tryptophane. The significance of phosphatase in the metabolism of reproduction in plants is indicated by the data of Ignatieff[9] who found that the phosphatase activity and the total phosphorous content of pistils and stamens are greater than in any other part of the flowering plant. In plant tissues other than leaves the inactive growth hormones may be true storage forms as indicated by the investigations of Gordon[6] on the hormone and protein combinations in wheat grains and the investigations of Berger and Avery[2] on the inactive hormone in maize endosperm. The possibility thus exists that the pollen substance is a coenzyme or activator of enzymatic systems present in the ovary which liberate active hormones from the storage forms.

*Summary.*—1. Determinations of growth hormone concentrations in pollen grains and pollen tubes have shown that free hormones are present in the pollen grains and that variable amounts are present during pollen tube growth. Larger quantities are present in a bound form that can be activated by alkali hydrolysis in the pollen of *Nicotiana* and by both acid and alkali hydrolysis in the pollen of *Antirrhinum*. The growth hormones in the pollen of *Datura* are all present in the active form. Maximum yields obtained by various procedures are in agreement.

2. Little or no growth hormone is present in the active form in dried ovary tissue of *Nicotiana* and *Antirrhinum* but large quantities are present in an inactive form that can be activated most completely by incubation at pH 8.0 and less completely by acid or alkali hydrolysis. A water extract of *Nicotiana* pollen contains a substance or substances which can bring about the conversion of the inactive growth hormones in the dried ovary tissue of *Nicotiana* to the active forms in an acid medium *in vitro*.

3. Comparisons of the amounts of growth hormones contained in the

pollen of a normal pollination with the amounts of diffusible hormones which appear in the ovary immediately after pollination and fertilization and with the amounts of inactive hormones in the ovary indicate that the changes in growth hormone concentrations following fertilization are the result of a substance in the pollen other than the growth hormones. It is suggested that this substance is part of an enzyme system.

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## *NUTRITIONAL LIFE HISTORY AS INFLUENCED BY DIETARY ENRICHMENTS. II. BODY WEIGHT AND BODY CALCIUM IN CASES OF PROTEIN-ACCELERATED GROWTH**

BY HENRY C. SHERMAN AND CONSTANCE S. PEARSON

DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY

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In a previous paper[1] we have reported that when a diet of about minimal adequacy in protein and calcium content was enriched in protein by the addition of poultry meat, growth was accelerated but calcium retention did not keep pace. During the resulting period of rapid growth with relatively low calcium content of body a minority of the experimental animals showed symptoms suggestive of calcium deficiency, while the

majority showed no visible injury and continued to make increased gains in body weight up to the age of 84 days as shown by the tabulated data of our preceding paper.

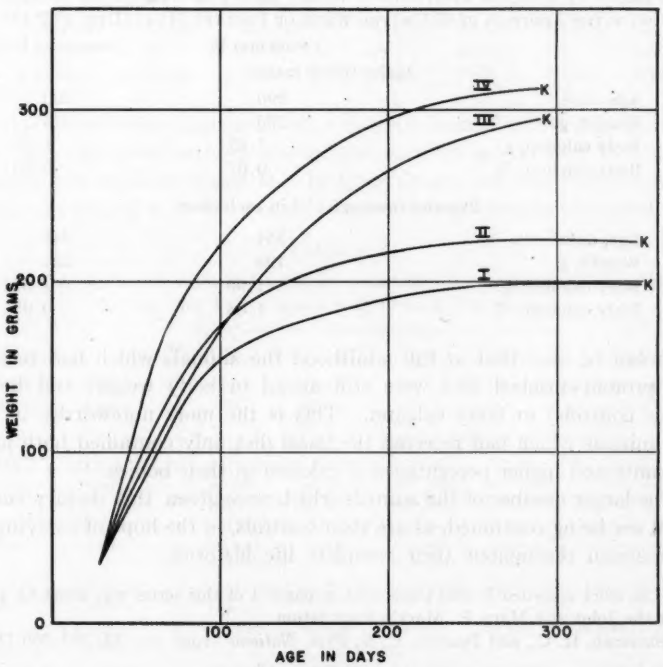


FIGURE 1

Smoothed curves representing body weights: I, average of three females receiving basal diet *ad lib;* II, average of three females receiving the same plus 60 g. per week of poultry meat; III, of a male receiving basal diet *ad lib;* IV, of a litter-mate male receiving the same plus 60 g. per week of poultry meat. The date of killing for analysis is indicated by K.

As the protein-accelerated rats reached about the average adult size for the animals of the Columbia colony, their weight curves flattened off while the smaller animals which had received the basal diet only, continued to grow. But at about one year of age when the slower-growing rats were also reaching their final adult size, the animals which had the protein-enriched diet still averaged larger than the controls which had received the basal diet only. This difference, while not large nor invariable, is probably significant. The curves shown in figure 1 are representative of our general experience on this point. These particular animals were then

killed and analyzed for body calcium at about one year of age, with the results shown in table 1.

TABLE 1

COMPARISON OF CALCIUM CONTENTS OF ADULT RATS FED BASAL DIET 16 ALONE OR WITH THE ADDITION OF 60 GM. PER WEEK OF POULTRY MEAT (DIET 16 P 10)

| | FROM DIET 16 | FROM DIET 16 P 10 |
|---|---|---|
| Males (litter mates) | | |
| Age, days | 290 | 290 |
| Weight, g. | 295 | 314 |
| Body calcium, g. | 1.87 | 1.76 |
| Body calcium, % | 0.67 | 0.60 |
| Females (average of 3 in each case) | | |
| Age, days | 354 | 348 |
| Weight, g. | 199 | 221 |
| Body calcium, g. | 1.99 | 1.89 |
| Body calcium, % | 1.08 | 0.93 |

It will be seen that at full adulthood the animals which had received the protein-enriched diet were still ahead in body weight and behind (their controls) in body calcium. This is the more noteworthy in that the animals which had received the basal diet only contained both larger amounts and higher percentages of calcium in their bodies.

The larger number of the animals which were given this dietary enrichment are being continued, as are their controls, in the hope of carrying the comparison throughout their complete life histories.

* The work recorded in this paper and in paper I of this series was aided by grants from the John and Mary R. Markle Foundation.

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## *DEPLETION MUTATION IN SACCHAROMYCES**

BY CARL C. LINDEGREN AND GERTRUDE LINDEGREN

THE HENRY SHAW SCHOOL OF BOTANY, WASHINGTON UNIVERSITY, ST. LOUIS, MO.

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An $\alpha$ mating type galactose-, maltose-fermenting haplophase segregant of *Saccharomyces cerevisiae* was subjected to mustard gas treatment by Tatum and Reaume (Tatum and Reaume, in ms.) and produced an adenine-dependent mutant with pink colonies. The symbol ad(P) indicates the adenine-dependent variant producing pink colonies; ad indicates the same allele carried by a white phenotype. The symbol AD indicates the dominant allele; no secondary symbol is necessary for

these are always white. The adenine-dependent pink (ad(P)) culture was shown to be a gene mutation by hybridizing it with an adenine-independent white (AD) haplophase of *S. cerevisiae.*

| | MATING | | HYBRID | SEGREGANTS | | | |
|---|---|---|---|---|---|---|---|
| | Haploid | × haploid | Diploid | Haploid | Haploid | Haploid | Haploid |
| Gene | ad(P) | × AD | ad(P)/AD | ad(P) | ad(P) | AD | AD |
| Color | Pink | × White | White | Pink | Pink | White | White |

Forty-six asci were dissected from the white hybrid and in 42 asci, two white and two pink cultures arose from each ascus, proving that gene mutation was involved (table 1). In these 42 asci the pink cultures were adenine-dependent and the white cultures were adenine-independent.

TABLE 1

GENETICAL ANALYSIS OF ASCI FROM VARIOUS CROSSES INVOLVING PINK AND WHITE COLOR, AND ADENINE AND METHIONINE SYNTHESIS

| MATINGS | NO. ASCI | SEGREGANTS A | B | C | D | COLOR |
|---|---|---|---|---|---|---|
| ad(P)MET × AD MET | 42 | ad(P) | ad(P) | AD | AD | 2 pink:2 white |
| pink white | 1 | ad(P) | ad | AD | AD | 1 pink:3 white |
| | 2 | ad | ad | AD | AD | 0 pink:4 white |
| | 1 | ad(P) | ad(P) | ad(P) | AD | 3 pink:1 white |
| ad(P)MET × AD met | 7 | ad(P)MET | ad(P)MET | AD met | AD met | 2 pink:2 white |
| pink white | 5 | ad met | ad met | AD MET | AD MET | 0 pink:4 white |
| | 23 | ad(P)MET | ad met | AD met | AD MET | 1 pink:3 white |
| ad met × AD MET | 7 | ad(P)MET | ad(P)MET | AD met | AD met | 2 pink:2 white |
| white white | 5 | ad met | ad met | AD MET | AD MET | 0 pink:4 white |
| | 27 | ad(P)MET | ad met | AD met | AD MET | 1 pink:3 white |
| ad(P)MET × ad(P)MET | 6 | ad(P)MET | ad(P)MET | ad(P)MET | ad(P)MET | 4 pink:0 white |
| pink pink | | | | | | |
| ad met × ad met | 3 | ad met | ad met | ad met | ad met | 0 pink:4 white |
| white white | | | | | | |
| ad(P)MET × AD MET | 7 | ad(P)MET | ad(P)MET | AD MET | AD MET | 2 pink:2 white |
| pink white | | | | | | |
| ad MET × ad(P)MET | 11 | ad(P)MET | ad(P)MET | ad(P)MET | ad(P)MET | 4 pink:0 white |
| white pink | | | | | | |
| ad MET × ad(P)MET | 10 | ad(P)MET | ad(P)MET | ad(P)MET | ad(P)MET | 4 pink:0 white |
| white pink | | | | | | |
| ad MET × ad met | 8 | ad(P)MET | ad(P)MET | ad met | ad met | 2 pink:2 white |
| white white | | | | | | |
| ad MET × AD met | 1 | ad(P)MET | ad(P)MET | AD met | AD met | 2 pink:2 white |
| white white | 5 | ad(P)MET | ad met | AD met | AD MET | 1 pink:3 white |

Three asci produced fewer than the expected number of pink segregants. One contained three white and one pink culture; the pink segregant and one white culture were adenine-dependent. Two asci produced 4 white cultures; two were adenine-dependent and two were adenine-independent. These exceptional adenine-dependent white cultures will be discussed in detail below.

From one ascus more than the expected number of pink cultures was

obtained (three pink and one white culture) and the three pink segregants were adenine-dependent. Most of the adenine-dependent cultures adapt after four days and grow in the adenine-deficient medium. The differences in growth in adenine-deficient and adenine-containing medium are generally diagnostic on the second and third days; but growth of the so-called adenine-dependent cultures in the adenine-deficient medium becomes fairly dense on the sixth and seventh day. The extra pink culture may have been slow in adapting to adenine synthesis due to some other deficiency.

The hybrid was also heterozygous for mating type, galactose-, maltose- and melibiose-fermentation and there were no exceptions of these characters to Mendelian segregation.

The hybrid from which the 46 asci were analyzed was homozygous for genes controlling the synthesis of methionine, so that relatively adequate amounts of methionine were available to all four segregants. Tatum and Reaume also discovered a methionine-dependent mutant produced by mustard gas treatment and this gene was introduced into the stock by a series of matings. A hybrid heterozygous for adenine-dependence and methionine-dependence was produced. Analysis of 74 asci from the reciprocal crosses ad(P) MET × AD met (pink × white) and ad met × AD MET (white × white) revealed that the development of pink color required methionine. The adenine-dependent *white* cultures were also methionine-dependent and all the adenine-dependent *pink* cultures were methionine-independent proving that methionine was required for the development of the pink color.

I have pointed out that a doubly heterozygous hybrid produces only three kinds of asci and that the frequency of these three types can be used to detect linkage of either of the genes with each other or with their respective centromeres. The data in table 1, show that the two reciprocal matings, one producing 35 and the other producing 39 asci, both follow the same pattern and the three kinds of asci are present with a total frequency of 14:10:50, which is statistically equivalent to a 1:1:4 ratio, proving that the two genes are not linked. Asci containing 2 pink:2 white; 0 pink:4 white; 1 pink:3 white, correspond to the same three categories and reveal that pink is produced as a result of a two-factor interaction.

Six asci from a pink by pink hybrid produced four pink cultures from each ascus. Three asci dissected from a homozygous adenine-dependent, methionine-dependent hybrid produced only white cultures. Seven asci from an ad(P)MET × AD MET back-cross hybrid produced 2 pink and 2 white per ascus. This further confirms the regular segregation of the two genes.

Adenine-dependence is the effect of the action of a single gene; pink pigment is a correlated effect which depends on the synergistic effect of

other genes as well. Pink pigment is apparently produced following the interaction of a precursor of adenine and an excess of methionine plus other substances. Pigment is usually produced in organisms incapable of completing the synthesis of adenine and capable of producing a considerable amount of methionine. The variation in intensity of color in different pink organisms indicates that many other factors affect color intensity.

*False Mutations.*—Some of the adenine-dependent, methionine-dependent *white* cultures were transferred to peptone agar to which an excess of methionine had been added. Pink cultures appeared thus confirming the dependence of the pink character on the presence of methionine. Added methionine did not induce the development of a pink color in any of the adenine-independent white organisms. When the cultures arising from homozygous ad met stocks were grown on agar, numerous small secondary pink papillae often appear suggesting local accumulation of sufficient methionine to produce the pink color.

Variations in bacteria following environmental changes have often been called "mutations" but the present experiments show that variations may also be due to a deficiency either of external or internal origin which prevents the development of the characteristic phenotype on a deficient medium. False "mutations" from pink to white may appear when growth occurs in the absence of sufficient methionine to insure the production of the pink color; many pink cultures have white borders which may arise when the supply of methionine in the medium becomes exhausted. Transfer of these false "mutants" to a medium containing sufficient methionine may result in a false "reverse mutation" from white to pink without any change occurring in the gene itself.

*Depletion Mutation.*—Tatum and Reaume discovered that white (ad MET) variant colonies which retain their methionine synthesizing ability often arise from pink cultures on vegetative propagation—a fact which we have confirmed, and which they will discuss in greater detail elsewhere. Numerous white variants of the original pink have appeared. These white variants are ad MET like the white variants of Tatum and Reaume. Similar white segregants arising in the pink pedigree were subjected to genitical analysis.

Two hybrids of one of the exceptional adenine-deficient methionine-sufficient (ad MET) *white* cultures (obtained among the 46 asci from the first hybrid in table 1) by standard pinks, ad(P)MET, produced 21 asci containing 4 pink cultures each. Eight asci from a hybrid of the exceptional white (ad MET) by a standard adenine-dependent, methionine-dependent white produced two pink and two white cultures per ascus; the white cultures were methionine-dependent. Six asci of an exceptional white (ad MET) by an adenine-independent, methionine-dependent white

produced the progeny that would be expected if the exceptional white were a normal pink. This analysis indicates that the inability of these exceptional adenine-dependent, methionine-independent cultures to produce the pink pigment was due to some mechanism which is restored to activity following hybridization.

The following hypothesis is invoked to explain the effect of outcrossing in restoring the pink color. Pink depends upon the presence of the two genes *ad* and *MET* plus some other substances (*X*, *Y*, *Z*, etc.). The substance *X* is an essential component of gene *X* which has no other components besides *X*. Continuous production of pink exhausts the supply of *X* and results in the "running out" of the character. The stock to which the outcross is made carries gene *X* with an intact supply of the *X* component for since the stock does not produce pink it does exhaust its supply of the *X* substance. The outcross automatically restores the *X* substance and reëstablishes the pink color. Other stocks may become white because *Y* or *Z* substances are exhausted. Mutations from pink to white are not the result of a drastic change in genotype but merely the result of the exhaustion of some gene component easily supplied by outcrossing to any normal stock.

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## *WIND-DRIVEN CURRENTS IN A BAROCLINIC OCEAN; WITH APPLICATION TO THE EQUATORIAL CURRENTS OF THE EASTERN PACIFIC**

By H. U. Sverdrup

Scripps Institution of Oceanography, University of California, La Jolla, California

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*1. Introduction.*—Permanent ocean currents are computed from the observed distribution of density on the assumptions (1) that the horizontal pressure gradient is balanced by the Coriolis force (the deflecting force of the earth's rotation) and (2) that the horizontal velocities and the hori-

zontal pressure gradient vanish at a moderate depth below the sea surface. The second condition can be fulfilled only in a baroclinic system, that is, in a system in which the isosteric surfaces intersect the isobaric surfaces.

In the computation of currents acceleration and frictional forces are neglected. Experience indicates that the computations lead to nearly correct results, implying that accelerations and frictional forces are small, but since friction is not entirely lacking, energy must be supplied to the ocean in order to maintain the permanent currents and the corresponding permanent distribution of mass. This energy can be supplied by the effects of heating and cooling or by the stress which the prevailing winds exert on the sea surface. Of the sources the latter is generally considered to be the more important. We shall examine effects of the wind stress only, taking into account that the ocean waters in motion represent a baroclinic system.

Ekman[1] and Stockmann[2] have examined the currents which develop in a *homogeneous* ocean under the influence of a stress exerted on the free surface, and Fjeldstad[3] has solved a special problem dealing with baroclinic conditions. If the general problem for a baroclinic ocean could be solved, knowledge of the wind stress alone would enable us to compute the permanent ocean currents, provided the effects of heating and cooling were negligible. A treatment of this general problem would present great mathematical difficulties because it would require the introduction of lateral frictional stresses and complete boundary conditions. Here we shall deal with the special case of equatorial currents in a region where lateral stresses can be neglected, boundary conditions are relatively simple, wind systems are semipermanent, and where our results imply that effects of heating and cooling, if present, need not be considered explicitly.

The striking feature of the currents of the equatorial regions is that imbedded between the currents which flow toward the *west* under the influence of the prevailing trade winds equatorial counter currents flow toward the *east*. In the Pacific and Atlantic Oceans the counter current is particularly well developed in the eastern parts of the oceans where it is located north of the equator, its axis coinciding approximately with the location of the equatorial calm belt which is found further to the north in summer than in winter. In the Indian Ocean the counter current is found to the south of the equator, but in the northern winter only.

Our specific problem is to determine whether the equatorial currents, including the counter currents, can be accounted for on the basis of our knowledge of the wind stress only. This problem was first approached by Montgomery and Palmén,[4] but Stockmann[2] has shown that they did not treat it in a sufficiently general manner. Stockmann's theoretical results, however, are not applicable to the conditions in the ocean because he assumed homogeneous water, but a similar analysis for a baroclinic

system leads to a remarkable agreement between theoretical conclusions and observed conditions.

*2. Theory.*—The ocean waters are so nearly in hydrostatic equilibrium that at any depth the pressure, $p$, can be determined by a numerical integration of the hydrostatic equation:

$$dp = g\rho\, dz \tag{1}$$

provided that the density, $\rho$, is known from observations. In equation (1) and in the following equations the $z$-axis is positive downwards.

Neglecting lateral stresses the equations of horizontal motion can be written:

$$\begin{aligned} \frac{\partial u}{\partial t} + u\frac{\partial u}{\partial x} + v\frac{\partial u}{\partial y} &= -\frac{1}{\rho}\frac{\partial p}{\partial x} + \lambda v + \frac{1}{\rho}\frac{\partial}{\partial z}\left(A\frac{\partial u}{\partial z}\right) \\ \frac{\partial v}{\partial t} + u\frac{\partial v}{\partial x} + v\frac{\partial v}{\partial y} &= -\frac{1}{\rho}\frac{\partial p}{\partial y} - \lambda u + \frac{1}{\rho}\frac{\partial}{\partial z}\left(A\frac{\partial v}{\partial z}\right) \end{aligned} \tag{2}$$

where $u$ and $v$ are the horizontal velocity components in a rectilinear coordinate system, $\lambda = 2\omega \sin \varphi$ ($\omega$ the earth's angular velocity of rotation, $\varphi$ the latitude, taken positive to the north of the equator), and $A$ is the eddy viscosity.

We shall assume stationary conditions,

$$\frac{\partial u}{\partial t} = \frac{\partial v}{\partial t} = 0, \tag{3}$$

and shall neglect the non-linear terms, the field accelerations:

$$u\frac{\partial u}{\partial x} + v\frac{\partial u}{\partial y} = u\frac{\partial v}{\partial x} + v\frac{\partial v}{\partial y} = 0, \tag{4}$$

thereby placing severe restrictions upon the possible lateral boundary conditions. Equations (2) reduce to:

$$\begin{aligned} \frac{\partial p}{\partial x} &= \lambda\rho v + \frac{\partial}{\partial z}\left(A\frac{\partial u}{\partial z}\right) \\ \frac{\partial p}{\partial y} &= -\lambda\rho u + \frac{\partial}{\partial z}\left(A\frac{\partial v}{\partial z}\right) \end{aligned} \tag{5}$$

stating that the horizontal pressure gradient is balanced by the Coriolis force and frictional stresses exerted on horizontal surfaces. In *homogeneous* water the horizontal pressure gradient is independent of depth but in a *baroclinic* system varies with depth. In the ocean it generally vanishes at

a moderate depth, less than that to the bottom. We define a function $P$ by the integrals

$$\frac{\partial P}{\partial x} = \int_0^d \frac{\partial p}{\partial x} dz, \qquad \frac{\partial P}{\partial y} = \int_0^d \frac{\partial p}{\partial y} dz \tag{6}$$

where $d$ is equal to or greater than the depth at which the horizontal pressure gradient becomes zero. The function $P$, which is closely related to the $P$-function introduced by Ekman,[5] can be computed from the observed vertical distribution of density at a single oceanographic station, using equation (1).

The horizontal velocity must vanish at or above the depth $d$. The integrals

$$M_x = \int_0^d \rho u dz, \qquad M_y = \int_0^d \rho v dz \tag{7}$$

represent therefore the components of the *net* mass transport by the currents.

Integrating equations (5) from 0 to $d$, and introducing the horizontal boundary conditions:

$$\begin{aligned} \left(A\frac{\partial u}{\partial z}\right)_0 &= -\tau_x, & \left(A\frac{\partial u}{\partial z}\right)_d &= 0 \\ \left(A\frac{\partial v}{\partial z}\right)_0 &= -\tau_y, & \left(A\frac{\partial v}{\partial z}\right)_d &= 0 \end{aligned} \tag{8}$$

where $\tau_x$ and $\tau_y$ are the components of the wind stress, we obtain:

$$\frac{\partial P}{\partial x} = \lambda M_y + \tau_x \tag{9a}$$

$$\frac{\partial P}{\partial y} = -\lambda M_x + \tau_y \tag{9b}$$

The terms in equations (9) are well known in oceanography. Omitting the stress components the equations give the mass transport related to the distribution of density, or assuming homogeneous water in hydrostatic equilibrium ($\partial P/\partial x = \partial P/\partial y = 0$) they give the mass transport by pure wind currents. Equations (9) have been used by Defant[6] for computing the wind stress from oceanographic observations, including direct measurements of currents, but they have not been applied to other problems.

For application to other problems we add the equation:

$$\frac{\partial M_x}{\partial x} + \frac{\partial M_y}{\partial y} = 0 \tag{10}$$

which is obtained by integration of the equation of continuity, assuming

that the vertical velocity is zero at the free surface and at the depth $d$. The three equations (9$a$), (9$b$) and (10) can be considered as relating the three unknown quantities, $P$, $M_x$, and $M_y$, to the known wind stress. Consequently, the distribution of density, as described by the partial derivatives of $P$, and the mass transport by the corresponding currents can be expressed as functions of the stress.

In applying equations (9) and (10) to equatorial currents we place the positive $x$-axis toward the east and the positive $y$-axis toward the north, and let $y = 0$ at the equator ($\varphi = 0$). Since

$$dy = R\,d\varphi \tag{11}$$

where $R$ is the radius of the earth:

$$\frac{\partial \lambda}{\partial x} = 0, \qquad \frac{\partial \lambda}{\partial y} = \frac{2\omega \cos \varphi}{R}, \qquad \frac{\partial^2 \lambda}{\partial y^2} = -\frac{2\omega \sin \varphi}{R^2} \tag{12}$$

Differentiating equation (9$a$) with respect to $y$ and (9$b$) with respect to $x$, subtracting and taking equations (10) and (12) into account, we obtain

$$M_y \frac{\partial \lambda}{\partial y} + \left(\frac{\partial \tau_x}{\partial y} - \frac{\partial \tau_y}{\partial x}\right) = 0 \tag{13}$$

In the trade-wind belt of the eastern Pacific the term $\partial \tau_y / \partial x$ is so small that with good approximation:

$$M_y = \frac{\partial \tau_x}{\partial y} \Big/ \frac{\partial \lambda}{\partial y} = -\frac{\partial \tau_x}{\partial y} \frac{R}{2\omega \cos \varphi} \tag{14}$$

Introducing equation (14) in (9$a$):

$$\frac{\partial P}{\partial x} = -\frac{\partial \tau_x}{\partial y} R \tan \varphi + \tau_x \tag{15}$$

or, writing differences on the left-hand side:

$$\frac{\Delta P}{\Delta x} = -\frac{\overline{\partial \tau_x}}{\partial y} R \tan \varphi + \bar{\tau}_x \tag{16}$$

where averages over the distance $\Delta x$ are indicated by bars.

From equations (10) and (14) follows

$$\frac{\partial M_x}{\partial x} = \frac{1}{2\omega \cos \varphi} \left(\frac{\partial \tau_x}{\partial y} \tan \varphi + \frac{\partial^2 \tau_x}{\partial y^2} R\right) \tag{17}$$

When integrating equation (17) from 0 to $\Delta x$ we shall assume a north-south vertical boundary at $x = 0$ at which the kinematic boundary condition $u_0 = 0$ must be satisfied in the form $M_0 = 0$. We obtain:

$$M_x = \frac{\Delta x}{2\omega \cos \varphi} \left( \overline{\frac{\partial \tau_x}{\partial y}} \tan \varphi + \overline{\frac{\partial^2 \tau_x}{\partial y^2}} R \right) \qquad (18)$$

Equation (18) cannot hold at a second north-south boundary at, say, $x = L$, at which the condition $M_L = 0$ must be satisfied. This inadequacy of our solution is due to the neglect of the field accelerations (eq. 4). Attempts will be made to find more general solutions and to study other special cases.

Substituting equation (18) in (9*b*):

$$\frac{\partial P}{\partial y} = -\Delta x \tan \varphi \left( \overline{\frac{\partial \tau_x}{\partial y}} \tan \varphi + \overline{\frac{\partial^2 \tau_x}{\partial y^2}} R \right) + \tau_y \qquad (19)$$

Equations (15) or (16) and (19), together with (14) and (18), represent in our special case the relationships of the distribution of mass and the corresponding mass transport to the wind stress. The validity of our results can be tested where suitable observations are available.

*3. Discussion.*—The available oceanographic observations comprise (1) a line of 8 stations between latitudes 22°N and 10°S, longitudes 137°W and 162°W, occupied by the *Carnegie* between October 21 and November 4, 1929 (Fleming[7]); (2) a line of 12 stations between latitudes 6°N and 9°S, longitudes 80°W and 108°W, occupied by the *Carnegie* between October 26 and November 21, 1928; and (3) a line of 8 stations between latitudes 9°N and 21°N, longitudes 87°W and 109°W, occupied by the Bushnell between March 18 and March 24, 1939 (Sverdrup[8]). From the observations at each of these stations the value of the function $P$ was computed by integrating to a depth of 1000 meters. From all data the ratio $\Delta P/\Delta x$ was found, and from the *Carnegie* section in mid-ocean $\partial P/\partial y$ was derived.

Wind observations comprise (1) monthly wind roses for 5-degree squares published in the Pilot Charts of the North and South Pacific, giving the percentage of winds from different directions and the corresponding average wind force (on the Beaufort scale) and (2) compilations of frequencies of winds of different forces in the "Atlas of Climatological Charts of the Oceans."[9] From the wind data the average wind stresses in October and November were computed, using the relationship

$$\tau = \gamma^2 \rho' U^2$$

where $\gamma^2$ is the resistance coefficient, $\rho'$ the density of the air, and $U$ the wind speed as estimated at a height of about 10 meters. At wind force 3 Beaufort or less the sea surface was assumed to be hydrodynamically smooth, with a resistance coefficient of about $0.8 \times 10^{-3}$, decreasing somewhat with increasing wind speed. At wind force 4 Beaufort and higher a constant value, $\gamma^2 = 2.6 \times 10^{-3}$, was used, corresponding to a hydrodynamically rough surface (Rossby[10]). The manner in which all computa-

tions were carried out will be described elsewhere by the author and R. O. Reid, who has prepared the figures in this paper.

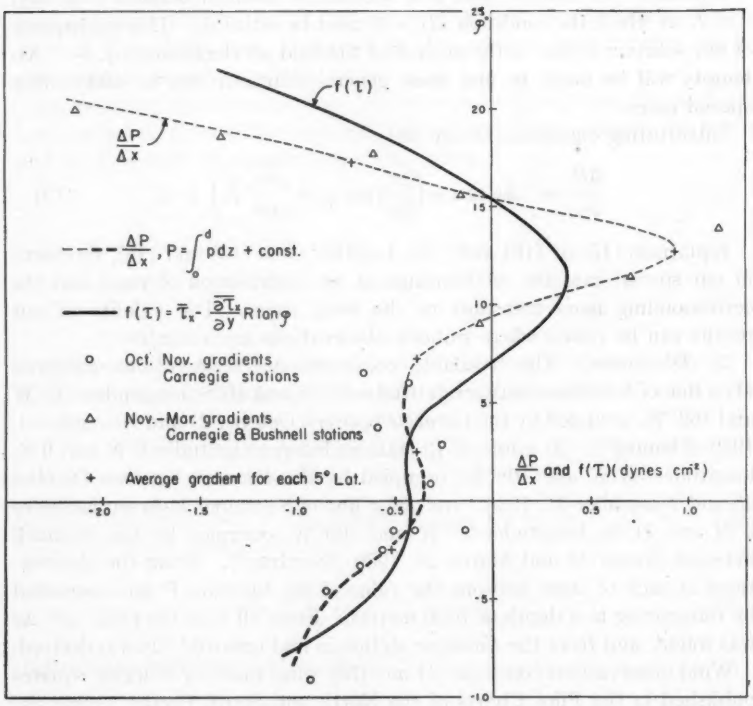


FIGURE 1

In figure 1 the terms of equation (16) are shown as functions of latitude. The curve that represents the left-hand term is heavily dashed to the south of latitude 6°N where the oceanographic observations upon which it is based were all taken in October–November, although in different years. To the north of 6°N the curve is shown by light dashes because observations off the American west coast in March have been combined with observations in mid-ocean in October. The right-hand term, the stress function, is shown by a full-drawn curve and is based on climatological wind data for the months October–November. The agreement between the curves is very good, considering that results of average wind conditions are compared with results derived from a few oceanographic stations which have been occupied in different seasons.

In figure 2 the $P$ function and the terms of equation (19) are plotted against latitude. The $P$ function is based on the *Carnegie* observations

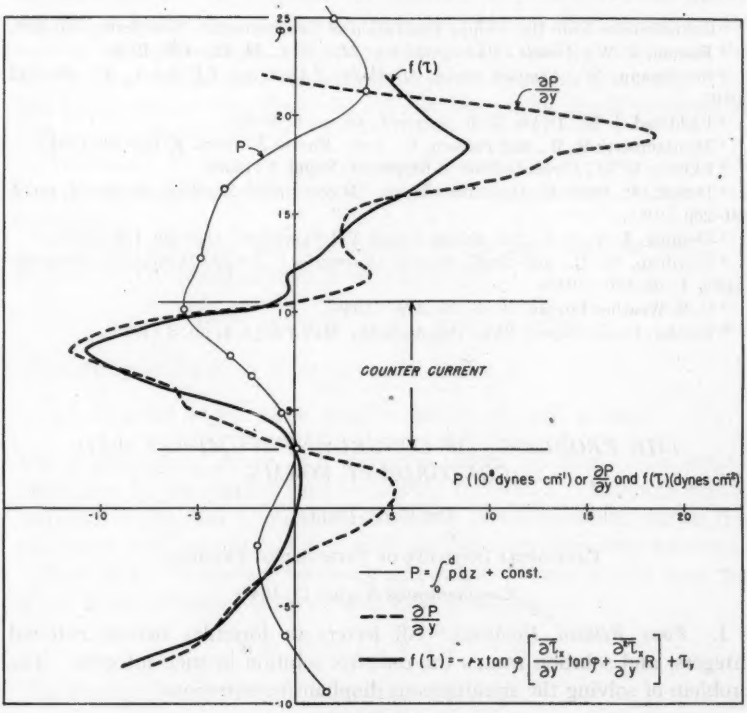


FIGURE 2

in mid-ocean in October–November, 1928, and the stress function on the average wind conditions in October–November over the ocean from the American west coast to the *Carnegie* section. A good agreement is obtained between the results based on a single oceanographic section and those derived from climatological wind charts.

*4. Conclusions.*—The distribution of density and the mass transport by the accompanying currents of the eastern equatorial Pacific depend entirely upon the average stress exerted on the sea surface by the prevailing winds. This conclusion is probably valid for the equatorial currents of all oceans but it has been demonstrated only for a case in which the non-linear terms in the equation of motion can be neglected.

It appears possible that the analysis of the relationship between wind stress and prevailing currents, assuming baroclinic conditions, can be

extended to other cases and can be developed into a powerful tool for examining permanent currents as well as changes produced by changing winds. Efforts in this direction are being continued.

* Contributions from the Scripps Institution of Oceanography, New Series, No. 324.

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## *THE PROBLEMS OF CONGRUENT NUMBERS AND CONCORDANT FORMS*

BY E. T. BELL

CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA

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*1. Four Related Problems.*—All letters in formulas denote rational integers, and solution means the *complete* solution in such integers. The problem of solving the simultaneous diophantine equations

$$rX^2 + mY^2 = rZ^2, \quad sX^2 + nY^2 = sW^2$$

includes as special cases two classical problems.

*Problem 1.*—If $r = s = Y^2 = 1$, $n = -m$, where $m$ is a given constant, the problem is that of congruent numbers. It goes back to Diophantus in the third century, the Arabs of the tenth and eleventh centuries, and Leonardo of Pisa (Fibonacci) in the early thirteenth century. For $m$ arbitrarily assigned it is still unsolved.

*Problem 2.*—For $r = s = 1$ the problem is Euler's (1780) of concordant forms, also unsolved.

Many special cases of these two have been investigated. Thus Fermat proved by his method of descent that if $m = n = -1$ in Problem 2, there are no integers $X$, $Y$, $Z$, $W$ all different from zero satisfying the equations. From this his theorem for fourth powers follows. Modern work originating in these problems has been concerned with cubics and quartics having

at most a finite number of sets of values of the indeterminates satisfying the equations. Some of this has used the theory of the units in special algebraic number rings. From the results it is possible, by the method applied to Problem 3, to derive much information on new diophantine systems of degrees higher than the second. This will be considered elsewhere. For the present, the inherent complexity of the solution of Problem 3 may suggest why these two old and apparently simple problems are still not completely solved.

*Problem 3.*—To state necessary and sufficient forms of $r, m, s, n$ in order that there shall exist $X, Y, Z, W$ all different from zero satisfying the equations.

A special case that has been frequently discussed may be noted. In Problem 1, the required form of $m$ is given by

$$4m = xyz^2w^2(x^2 - y^2),\ w(x + y) \text{ even.}$$

The corresponding $X, Z, W$ are given by

$$4X = zw(x^2 + y^2),\ 4Z = zw(x^2 + 2xy - y^2),\ 4W = zw(x^2 - y^2).$$

For $m$ squarefree, $zw = \pm 1$, giving a known criterion. The proof is immediate by the method used for solving Problem 3. Although it is not included in Problem 3, another, somewhat similar problem, dating from the Arabs and usually included with questions on congruent numbers is

*Problem 4.*—To state a necessary and sufficient form of $n$ in order that $X, Y, Z$ all different from zero shall exist satisfying

$$n + X^2 = Y^2,\ n - X^2 = Z^2.$$

The solution is given by

$$4n = x^2(a^2y^4 + b^2z^4),\ ab = 2;$$

the corresponding $X, Y, Z$ are given by

$$X = xyz,\ 2Y = x(ay^2 + bz^2),\ 2Z = x(ay^2 - bz^2).$$

This is equivalent to

$$ab = 2,\ fgh^2 = 4,\ a^2y^4 + b^2z^4 = fu,\ n = guv^2;$$

$$X = ghyzv,\ 2Y = ghv(ay^2 + bz^2),\ 2Z = ghv(ay^2 - bz^2).$$

2. *Solution of Problem 3.*—If $r, m, s, n, X, Y, Z, W$ are indeterminates, the equations are homogeneous cubics, each of which is separable and hence (completely) solvable. The result of equating the parametric expressions for $X$ and those for $Y$ in the solutions gives a separable and hence (completely) solvable system. As the solution of separable equations,

or of a system of such equations, is now straightforward routine, it will suffice to state the final result. To condense the formulas, write

$$a \equiv a_1a_2a_3a_4a_5,\ b \equiv b_1b_2b_3b_4b_5,\ c \equiv c_1c_2c_3c_4c_5,$$
$$f \equiv f_1f_2f_3f_4f_5,\ g \equiv g_1g_2g_3g_4g_5,\ h \equiv h_1h_2h_3h_4h_5,$$
$$\alpha \equiv b_1c_1f_1g_1h_1,\ \beta \equiv a_1c_2f_2g_2h_2,\ \gamma \equiv a_2b_2f_3g_3h_3,$$
$$\theta \equiv a_3b_3c_3g_4h_4,\ \phi \equiv a_4b_4c_4f_4h_5,\ \psi \equiv a_5b_5c_5f_5g_5;$$
$$\pi \equiv abcfgh,\ m \equiv pm_1m_2,\ n \equiv tn_1n_2.$$

Thus $p$, $m_1$, $m_2$ are bound parameters whose product is $m$; similarly for $t$, $n_1$, $n_2$ and $n$. The $a_i, \ldots, g_i$ are independent parameters. Define

$$A \equiv m_1afg^2 - n_1\alpha\theta\phi^2,\ B \equiv m_2bfh^2 - n_2\beta\theta\psi^2,$$
$$C \equiv m_2n_1bh^2\alpha\phi^2 - m_1n_2ag^2\beta\psi^2,$$

introduce the parameters $x$, $y$, $z$ and define $e$, for assigned values of all the parameters, as an arbitrary integer multiple of the reciprocal of the greatest common divisor of

$$xy^2A,\ xz^2B,\ y^2z^2C.$$

(If $e$ is merely an arbitrary integer, the values of $r$, $s$, $X$, $Y$, $Z$, $W$ stated presently, with $p$, $m_1$, $m_2$, $t$, $n_1$, $n_2$ as above, satisfy the equations identically, but this does not exhaust the possibilities. The stated definition of $e$ is necessary.) Introduce the parameter $u$. The required values of $r$, $s$ are

$$r = e^2pu^2x^2y^2z^2abc^2AB,\ s = e^2tu^2x^2y^2z^2\alpha\beta\gamma^2AB.$$

To state the corresponding values of $X$, $Y$, $Z$, $W$ define

$$F \equiv m_2n_1bh^2\alpha\phi^2 - ag^2\beta\psi^2$$
$$G \equiv 2m_1m_2abfg^2h^2 - m_1n_2ag^2\beta\theta\psi^2 - m_2n_1bh^2\alpha\theta\phi^2,$$
$$H \equiv m_1n_2afg^2\beta\psi^2 + m_2n_1bfh^2\alpha\phi^2 - 2n_1n_2\alpha\beta\theta\phi^2\psi^2.$$

Introduce a parameter $k$. Then

$$2X = ekx^2y^2z^2f\theta F,\ Y = e^2kux^2y^2z^2\pi AB,$$
$$2Z = ekx^2y^2z^2fG,\ 2W = ekx^2y^2z^2\theta H.$$

Including the bound parameters $p$, $m_1$, $m_2$, $t$, $n_1$, $n_2$ there are in all 41. Each of $m$, $n$ is of degree 3; each of $r$, $s$, is of degree 71; each of $X$, $Z$, $W$ is of degree 49, and $Y$ is of degree 83. The degree of each of the identities giving the solution of the cubic system is thus 169.

# ON A THEOREM OF VON NEUMANN

BY LLOYD L. DINES

DEPARTMENT OF MATHEMATICS, SMITH COLLEGE

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In these PROCEEDINGS, August 15, 1946, Loomis[1] presented a simple proof of a theorem due to J. von Neumann[2, 3] and formulated by Loomis as follows:

*Let $a_{ij}$ and $b_{ij}$ be two rectangular matrices $(i = 1, \ldots, n)$, $(j = 1, \ldots, m)$ such that $a_{ij} > 0$ for all $i, j$. Then there exists a unique $\lambda$ and vectors $x = (x_1, \ldots, x_m)$, $y = (y_1, \ldots, y_n)$ subject to $x_j \geqq 0, y_i \geqq 0, \Sigma_1^m x_j = 1$ and $\Sigma_1^n y_i = 1$ such that*

$$\lambda \sum_{j=1}^{m} a_{ij}x_j \geqq \sum_{j=1}^{m} b_{ij}x_j, \quad i = 1, \ldots, n, \tag{1}$$

$$\lambda \sum_{i=1}^{n} a_{ij}y_i \leqq \sum_{i=1}^{n} b_{ij}y_i, \quad j = 1, \ldots, m. \tag{2}$$

The present note gives an alternative simple proof, the essence of which is a reduction of the question to consideration of a single system of linear *equations*. This reduction is accomplished in two steps: 1st, replacement of (1) and (2) by an equivalent pair of adjoint linear systems

$$\sum_{j=1}^{m} \alpha_{ij}x_j \geqq 0, \qquad i = 1, \ldots, n + m, \tag{1'}$$

$$\sum_{i=1}^{n+m} \alpha_{ij}y_i = 0, \qquad j = 1, \ldots, m; \tag{2'}$$

and 2nd, utilization of a known relationship[4] between adjoint systems of form (1'), (2'). This relationship is simply that *the system of inequalities (1') admits a solution $x$ which does not annul all the left members if, and only if, the system of equations (2') admits no positive solution $y$ (every $y_i$ positive).*

The system (1') consists of $n + m$ inequalities, the first $n$ of which are those in (1) after obvious transpositions, and the last $m$ are the conditions $x_j \geqq 0, j = 1, \ldots, m$, which occur as collateral conditions in the statement of the theorem.

The system (2') consists of $m$ *equations*, which are equivalent to the $m$ inequalities of (2) by virtue of the introduction of $m$ new and essentially *non-negative* variables $y_{n+1}, \ldots, y_{n+m}$.

The explicit definitions of the coefficients $\alpha_{ij}$ are

$$\alpha_{ij} = \lambda a_{ij} - b_{ij}, \qquad i = 1, \ldots, n; \qquad j = 1, \ldots, m,$$
$$\alpha_{ij} = \delta_{ij}, \qquad i = n + 1, \ldots, n + m; \qquad j = 1, \ldots, m,$$

where $\delta_{ij} = 1$ if $i = n + j$; otherwise it is *zero*.

In view of the relationships which have been indicated above, the von Neumann theorem will be justified if we show the existence of a unique value of the parameter $\lambda$ for which the system of equations (2′) admits no *positive* solution $y = (y_1, \ldots, y_{n+m})$, but does admit a *non-negative* solution with at least one $y_k$ positive.

To this end, we consider the explicit form of the system (2′), which may be written

$$\sum_{i=1}^{n} (\lambda a_{ij} - b_{ij}) y_i + y_{n+j} = 0, \qquad j = 1, \ldots, m. \qquad (2'')$$

Since $a_{ij} > 0$ for every $i, j$, it is obvious that for $\lambda$ sufficiently small the system will admit positive solutions $y$, while for $\lambda$ sufficiently large it will admit no such solution. Furthermore if it admits a positive solution for any particular value of $\lambda$, it will admit such a solution for any smaller value. We denote by $\lambda_0$ the least upper bound of the values of $\lambda$ for which (2″) admits a positive solution $y$. Since to every positive solution $y$ there corresponds by simple normalization a solution for which $\Sigma_1^{n+m} y_k = 1$, it follows from the compactness of the closed and bounded sub-space defined by $y_k \geqq 0$, $\Sigma_1^{n+m} y_k = 1$ that to the value $\lambda = \lambda_0$ there corresponds a solution of (2″) in this subspace; call it $y^\circ = (y_1^\circ, \ldots, y_{n+m}^\circ)$.

At least one of the coördinates $y_k^\circ$ must be *zero*. For if they were all positive, a sufficiently small $\epsilon > 0$ would make correspond to the parameter value $\lambda_0 + \epsilon$ a *positive* solution $y' = (y_1', \ldots, y_{n+m}')$ with $y_i' = y_i^\circ$, $(i = 1, \ldots, n)$, and $y_{n+j}' = y_{n+j}^\circ - \epsilon \Sigma_1^n a_{ij} y_i^\circ$, $(j = 1, \ldots, m)$, thus contradicting the definition of $\lambda_0$. In $\lambda_0$ we have therefore *one* value of the parameter $\lambda$ which affords an admissible solution.

No second value of $\lambda$ does. For if $\lambda_1 > \lambda_0$ did afford an admissible solution $y'$, then $\Sigma_1^n (\lambda_1 a_{ij} - b_{ij}) y_i' \leqq 0$, $(j = 1, \ldots, m)$, and consequently, for any $\epsilon > 0$

$$\sum_{i=1}^{n} [(\lambda_1 - \epsilon) a_{ij} - b_{ij}] y_i' < 0, \qquad j = 1, \ldots, m,$$

and from the continuity of the linear functions these inequalities remain strictly valid if each $y_i'$ be replaced by $y_i'' = y_i' + \eta_i$ with $\eta_i > 0$ and sufficiently small. Defining $y_{n+j}''$, $(j = 1, \ldots, m)$ by the equations

$$\sum_{i=1}^{n} [(\lambda_1 - \epsilon) a_{ij} - b_{ij}] y_i'' + y_{n+j}'' = 0, \qquad j = 1, \ldots, m,$$

we would then have a *positive* solution corresponding to the parameter value $\lambda_1 - \epsilon > \lambda_0$, thus contradicting the definition of $\lambda_0$.

This completes the proof of the theorem. The method of proof definitely suggests the possibility of generalization, since the vital relationship between the adjoint systems (1′) and (2′) persists when the finite matrix

$\alpha_{ij}$ is replaced by a function $\alpha(p, q)$ and the summations are replaced by more general linear operators.

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[2] von Neumann, J., "Über ein ökonomisches Gleichungssystem, etc.," *Ergebnisse eines Mathematischen Kolloquiums*, **8**, 73–83 (1937).

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[4] Dines, L. L., "Convex Extension and Linear Inequalities," *Bull. Amer. Math. Soc.*, **42**, 353–365 (1936).

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## *NOTE ON CONTINUOUS REPRESENTATIONS OF LIE GROUPS*

BY LARS GÅRDING

INSTITUTE OF MATHEMATICS, LUND, SWEDEN, AND PRINCETON UNIVERSITY

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Let $G$ be an analytic Lie group with unit element $e$ and let $a \to T(a)$, $(a \in G)$, be a representation of $G$ as bounded operators $T(a)$ on a Banach space $B$ such that for every $x \in B$, $T(e)x = x$ and $T(a)x \to T(b)x$ when $a \to b$. Let $A = (a(s))$, ($s$ real), be a one-parameter subgroup in $G$ and define

$$T_A x = \lim_{s \to 0} s^{-1}(T(a(s)) - 1)x$$

whenever the limit exists in which case $x$ is said to be in the domain of $T_A$. I. Gelfand proved that every such domain is dense in $B$.[1] By a slight extension of his method we will prove that they have a dense intersection when $A$ varies, thus answering a question left open by W. Wigner[2] and V. Bargmann.[3]

Let $r$ be a not-negative integer or $+\infty$, let $C_r$ be the class of real functions defined on $G$ with continuous derivatives of order $\leqslant r$, ($< r$ if $r = \infty$), and let $C_r^0$ be the subset of $C_r$ whose elements vanish outside a compact set and let $B_r$ be the set of elements of $B$ of the form

$$x(h) = \int_G h(b)T(b)x db \qquad (h \in C_r^0,\ x \in B),$$

where $db$ is a left invariant volume element on $G$.

THEOREM. *Every $B_{r+1}$ is dense in $B$, it is in the domain of any $T_A$ and $T_A B_{r+1}$ is contained in $B_r$ for any $A$.*

*Proof:* One has with $s \neq 0$

$$s^{-1}(T(a(s)) - 1)x(h) = s^{-1} \int_G h(b)(T(a(s)b) - T(b))x db = \int_G s^{-1}(h(a^{-1}(s)b) - h(b))T(b)x db.$$

Hence, when $s \to 0$

$$T_A x(h) = x(h_A),$$

where

$$h_A(b) = \lim_{s \to 0} s^{-1}(h(a^{-1}(s)b) - h(b)) \in C_r^0$$

if $h \in C_{r+1}^0$. This proves the second part of the theorem. To prove the first part we remark that it is possible to choose a sequence of not-negative functions $h_1, h_2, \ldots$ in $C_\infty^0$ such that $\int_G h_k(a)da = 1$ and for any open set $C$ containing $e$, $\int_{G-C} h_k(a)da = 0$ when $k$ is sufficiently large. Then $x(h_k) \in B_r$ and $x(h_k) \to x$ when $k \to \infty$ for any $x$ and $r$.

Similar theorems can be obtained for semigroups with a unit element and even for differentiable manifolds as follows.

Let $M$ be a $C_2$-manifold of $m$ dimensions in the sense of Whitney[4] with defining mappings $\theta_1, \theta_2, \ldots$ of the interior $Q$ of the unit $(m-1)$-sphere into $M$. Let $(p, q) \to r = f(p, q)$ be a mapping of class $C_1$ of $M \times M$ into $M$ which is locally one-to-one when $p$ and $q$ are fixed $(p, q, r \in M)$.

Let $p \to T(p)$ be a mapping of $M$ upon a set of bounded operators $T(p)$ on a Banach space $B$ which is continuous in the sense used above and is such that there exists a sequence of points $p_1, p_2, \ldots$ in $M$ such that $T(p_k)x \to x$ for every $x$ in $B$ when $k \to \infty$, and

$$T(p)T(q) = T(f(p, q)).$$

Let $p = \theta_k(a)$, $(a \in Q)$, and $0 \neq b \in Q$ and put

$$T_k(p, b)x = \lim_{s \to 0} s^{-1}(T(\theta_k(a + sb)) - T(\theta_k(a)))x$$

whenever the limit exists. *Then the domains of $T_k(p, b)$ have a dense intersection $I$.* In fact it is easily verified that the elements of $B$ of the form

$$x_j(h) = \int_Q h(t)T(\theta_j(t))x dt \qquad (x \in B),$$

belong to $I$ if $h(t)$ is of class $C_1$ and vanishes outside a compact subset of $Q$.

To prove that $I$ is dense in $B$ it suffices to take a sequence $j_1, j_2, \ldots$ such that $p_k \in \theta_{j_k}(Q)$ and suitable not-negative functions $h_1, h_2, \ldots$ in $C_1$ which vanish outside suitable open sets containing $\theta_{j_k}^{-1} p_k$ so that $x_{j_k}(h_k) \to x$ as $k \to \infty$.

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[2] Wigner, E., "On Unitary Representations of the Inhomogeneous Lorentz Group," *Annals of Math.*, **40**, 149–204 (1939).

[3] Bargmann, V., "Irreducible Representations of the Lorentz Group," *Ibid.*, **48**, 568–640 (1947).

[4] Whitney, H., "Differentiable Manifolds," *Ibid.*, **37**, 645 (1936).

# ON LACUNARY TRIGONOMETRIC SERIES

BY R. SALEM AND A. ZYGMUND

THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY AND THE UNIVERSITY OF CHICAGO

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Let us consider a lacunary trigonometric series

$$\sum_{k=1}^{\infty} (a_k \cos n_k x + b_k \sin n_k x), \text{ with } n_{k+1}/n_k > q > 1, \qquad (1)$$

and let us confine our attention to the interval $0 \leqslant x \leqslant 2\pi$. Let $S_N(x)$ denote the $N$th partial sum of (1), that is to say the sum of the terms with $k = 1, 2, \ldots, N$. Let $C_N = \left\{ {}^1/_2(a_1^2 + b_1^2 + \ldots + a_N^2 + b_N^2) \right\}^{1/2}$. In a recent note,[1] J. Ferrand and R. Fortet considered the behavior of the ratio $S_N(x)/C_N$. They stated, without proof, that if $C_N \to +\infty$ as $N \to \infty$, the distribution function of $S_N(x)/C_N$ tends to the Gaussian distribution with mean value 0 and dispersion 1. Clearly, the result as stated cannot be correct since, if $c_k = (a_k^2 + b_k^2)^{1/2}$ increases very rapidly, the distribution function tends to that of cosine. Thus some kind of restriction on the $c_k$ is necessary. (Mr. Erdös informs us that a proof of the result, under the condition $C_n \to \infty$, $c_n = O(1)$, will be published in a paper written jointly by him, Mlle. Ferrand, Fortet and Kac.) In this note we propose to give a complete solution of the problem.

By a distribution function we shall mean any non-decreasing and continuous to the right function $F(y)$ with $F(-\infty) = 0$, $F(+\infty) = 1$. Let $Z_n(y)$ be the set of points $x$ from $(0, 2\pi)$ at which $S_N(x)/C_N \leqslant y$. Let $F_N(y) = |Z_N(y)|/2\pi$ (by $|E|$ we mean the measure of the set $E$), so that $F_N$ is the distribution function of $S_N/C_N$.

(i) *If $F_N(y)$ tends to a distribution function $F(y)$* (at the points of continuity of the latter) *such that either $F(y) > 0$ or $F(y) < 1$ for all finite $y$, then*

$$c_n/C_n \to 0. \qquad (2)$$

(ii) *If (2) is satisfied and if $C_n \to \infty$, then $F_N(y)$ tends to the Gaussian distribution with mean value 0 and dispersion 1.*

We shall even prove a more general result, namely, that the distribution function of $S_N(x)/C_N$ on every fixed set of positive measure tends to the Gaussian distribution.

(iii) *Let $E$ be a point set on $(0, 2\pi)$, with $|E| > 0$, and let $F_N(y; E) = |Z_N(y)E|/|E|$. If $C_n \to \infty$ and if (2) holds, then $F_N(y; E)$ tends to the Gaussian distribution with mean value 0 and dispersion 1.*

To prove (i), let us assume for example that $F(y) < 1$ for all finite $y$, and that (2) is false. Hence $c_N/C_N > \sqrt{2}\epsilon > 0$ for infinitely many $N$.

Let us confine our attention to such $N$. Obviously, $C_{N-1}/C_N < (1-\epsilon^2)^{1/2}$, and the formula,

$$\frac{S_N}{C_N} = \frac{S_{N-1}}{C_{N-1}} \cdot \frac{C_{N-1}}{C_N} + \frac{a_N \cos n_N x + b_N \sin n_N x}{C_N},$$

shows that at every point $x$ from $Z_{N-1}(y)$, $y > 0$, we have $S_N(x)/C_N \leqslant y(1-\epsilon^2)^{1/2} + \sqrt{2}$. It follows that $Z_{N-1}(y)$ is included in $Z_N(y(1-\epsilon^2)^{1/2} + \sqrt{2})$, and so $F_{N-1}(y) \leqslant F_N(y(1-\epsilon^2)^{1/2} + \sqrt{2})$. Let $y$ be a point of continuity of $F$. Making $N \to \infty$, we get $F(y) \leqslant F(y(1-\epsilon^2)^{1/2} + \sqrt{2})$. However, from the assumption that $F(y)$ is always $<1$ we obtain that there are points $y > 0$ of continuity of $F$ at which $F(y(1-\epsilon^2)^{1/2} + \sqrt{2}) < F(y)$. This contradiction completes the proof of (i). Condition $C_N \to \infty$ and condition (2) imply that $\mathrm{Max}_{1 \leq k \leq N} c_k/C_N \to 0$ as $N \to \infty$. This remark will be used repeatedly below.

To prove (iii), let us assume for the sake of brevity that (1) is a purely cosine series, and let us write $A_N$ for $C_N$ (in the general case we merely represent the terms of (1) in the form $c_k \cos(n_k x + \theta_k)$ and the argument that follows remains unchanged). It is enough to prove that over any finite range of $\lambda$ the characteristic function of $F_N(y; E)$ uniformly approaches that of the Gaussian distribution. The characteristic function of $F_N(y; E)$ is

$$\int_{-\infty}^{+\infty} e^{i\lambda y} dF_N(y; E) = |E|^{-1} \int_E e^{i\lambda S_N(x)/A_N} dx =$$

$$|E|^{-1} \int_E \exp\left\{i\lambda A_N^{-1} \sum_1^N a_k \cos n_k x\right\} dx =$$

$$|E|^{-1} \int_E e^{o(1)} \prod_{k=1}^N \left(1 + \frac{i\lambda a_k}{A_N} \cos n_k x\right) \exp\left\{-\tfrac{1}{2} \frac{\lambda^2 a_k^2}{A_N^2} \cos^2 n_k x\right\} dx, \quad (3)$$

on account of (2) and of the relation $\exp. z = (1+z)\exp.\{\tfrac{1}{2}z^2 + o(|z|^2)\}$ valid for $z \to 0$. Due to (2) and $A_N \to \infty$, the term $o(1)$ in $e^{o(1)}$ tends to 0 uniformly in $x$ as $N \to \infty$ (provided $\lambda = O(1)$, an assumption we always make from now on). Let us now observe that

$$\left|\prod_{k=1}^N \left(1 + \frac{i\lambda a_k}{A_N} \cos n_k x\right)\right| \leqslant \prod_{k=1}^N \left(1 + \frac{\lambda^2 a_k^2}{A_N^2}\right)^{1/2} \leqslant e^{\gamma^2}, \quad (4)$$

and that

$$\sum_{k=1}^N \frac{a_k^2}{A_N^2} \cos^2 n_k x = 1 + \sum_{k=1}^N \frac{a_k^2}{2A_N^2} \cos 2n_k x = 1 + \xi_N(x).$$

The measure of the set of points where $|\xi_N(x)| \geqslant \delta > 0$ is less than $\delta^{-2} \int_0^{2\pi} \xi_N^2(x) dx = \tfrac{1}{4}\pi\delta^{-2}(a_1^4 + \ldots + a_N^4)/A_N^4$, and so tends to 0 on account of (2) and $A_N \to \infty$. Since $|\xi_N(x)| \leqslant 1$, it follows that, with an error tending uniformly to 0 as $N \to \infty$, the integral (3) is

$$|E|^{-1}e^{-\lambda^2/2}\int_E \prod_{k=1}^{N}\left(1+\frac{i\lambda a_k}{A_N}\cos n_k x\right)dx = |E|^{-1}e^{-\lambda^2/2}I_N, \quad (5)$$

and it is enough to prove that $I_N$ tends uniformly to $|E|$. We shall write

$$\prod_{1}^{N}\left(1+i\lambda\frac{a_k}{A_N}\cos n_k x\right) = \alpha_0^{(N)} + \sum_{1}\alpha_\gamma^{(N)}\cos\gamma x,\ \epsilon_k = \epsilon_k^{(N)} = |i\lambda a_k/A_N|, \quad (6)$$

so that $\alpha_\gamma^{(N)}$, $\epsilon_k$ depend on $\lambda$ also.

If $q \geqslant 3$, the proof of $I_N \to |E|$ is simple. For the numbers $\alpha_\gamma^{(N)}$ which actually occur in (6) correspond to the indices $\gamma \geqslant 0$ of the form $\pm n_{k_1} \pm n_{k_2} \pm \ldots \pm n_{k_p}(k_1 > k_2 > \ldots > k_p)$. For $q \geqslant 3$ such a representation (if it exists) is unique. In particular, $\alpha_0^{(N)} = 1$. Thus $I_N = |E| + \pi\sum h_\gamma\alpha_\gamma^{(N)}$, where $h_\gamma$ are the cosine Fourier coefficients of the characteristic function of $E$. On account of (2), each $\alpha_\gamma^{(N)}$ tends to 0 as $N \to \infty$ and $\gamma = 1, 2, \ldots$. Thus, if $\gamma_0$ is fixed,

$$\sum_{1}|h_\gamma\alpha_\gamma^{(N)}| \leqslant \sum_{1}^{\gamma_0} + \sum_{\gamma_0+1} \leqslant o(1) + \Big(\sum_{\gamma_0+1}^{\infty} h_\gamma^2\Big)^{1/2}\Big(\sum_{\gamma_0+1}|\alpha_\gamma^{(N)}|^2\Big)^{1/2}. \quad (7)$$

The first factor in the last product is arbitrarily small if $\gamma_0$ is large enough (since $\sum h_\gamma^2 < \infty$), and the last factor is bounded by, (6), (4), and Parseval's formula. Thus $I_N \to |E|$, and (iii) follows. (If $E = (0, 2\pi)$, then even the weaker condition $q \geqslant 2$ implies that $\alpha_0^{(N)} = 1$, so that $I_N = 2\pi$, and (ii) follows.)

The proof of the general case of (iii) is based on two lemmas.

LEMMA 1. *The number of solutions of $n_{k_1} + n_{k_2} + \ldots + n_{k_p} = a$, where $a$ is given is less than $C^{p-1}$, where $C = C(q)$ depends on $q$ only.* For assuming that $k_1 > k_2 > \ldots > k_p$, we have $n_{k_1} \leqslant a \leqslant n_{k_1}\, q/(q-1)$, so that $n_{k_1}$ takes $\leqslant C(q)$ values. The same applies to $n_{k_2}$ when $n_{k_1}$ is fixed and so on.

LEMMA 2. *The number of solutions of $n_{k_1} + \ldots + n_{k_p} - n_{k_{p+1}} = a(k_1 > k_2 > \ldots > k_p > k_{p+1})$ is less than $C^p$, where $C$ is the same as in Lemma 1.* For $p = 1$ this is immediate since $n_{k_1} - n_{k_2} = a$ implies $n_{k_1}(1 - 1/q) \leqslant a \leqslant n_{k_1}$, and the ratio of the extreme terms here is the same as in the inequality for $a$ in the proof of Lemma 1. Combining this special case with Lemma 1 we get Lemma 2.

Let us now revert to the first formula (6). As the argument (7) shows, (iii) will be established when we show that $\alpha_0^{(N)} \to 1$, $\alpha_\gamma^{(N)} \to 0$ for $\gamma = 1, 2, \ldots$. As a matter of fact, we can prove that $\alpha_\gamma^{(N)} \to 0$ uniformly in $\gamma > 0$. The $\gamma$ actually occurring in (6) are $\geqslant 0$ and of the form $\pm n_{k_1} \pm n_{k_2} \pm \ldots \pm n_{k_p}$ $(k_1 > k_2 > \ldots > k_p)$. Let us fix $p$ and let us write the last equation in the form

$$n_{h_1} + n_{h_2} + \ldots + n_{h_s} = \gamma + n_{l_1} + n_{l_2} + \ldots + n_{l_t}. \quad (8)$$

with $p = s + t$, $h_1 > h_2 > \ldots > h_s$, $l_1 > l_2 > \ldots > l_t$. Let us also fix $s$ and $t$. Clearly, the case $s = t = 0$ leads to the contribution 1 in $\alpha_0^{(N)}$ so that, in order to estimate $\alpha_0^{(N)} - 1$, $\alpha_\gamma^{(N)}$ $(\gamma = 1, 2, \ldots)$ we must assume that $s \geqslant 1$, $t \geqslant 0$. For the clarity of presentation, let us first assume that $s > 1$, $t > 1$. Let us also assume that $h_s < l_t$. Thus we investigate the solutions of (8) under the conditions

$$\gamma \geqslant 0,\ s > 1,\ t > 1 - \text{fixed},\ h_s < l_t. \tag{9}$$

Every solution of (8) contributes to $\alpha_\gamma^{(N)}$ (or to $\alpha_0^{(N)} - 1$) as much as $\epsilon_{h_1} \ldots \epsilon_{h_s} \epsilon_{l_1} \ldots \epsilon_{l_t}/2^{p-1}$ in absolute value. The total contribution of the solutions of (8) satisfying (9) is therefore $\leqslant S = 2^{-(p-1)} \sum \epsilon_{h_1} \ldots \epsilon_{h_s} \epsilon_{l_1} \ldots \epsilon_{l_t}$, summation being extended over all indices satisfying (8) and (9). Let $\eta = \text{max.}\ (\epsilon_1, \epsilon_2, \ldots, \epsilon_N)$ so that $\eta = \eta_{N,\lambda} \to 0$ as $N \to \infty$, $\lambda = O(1)$. Then $S \leqslant 2^{-(p-1)} \eta \sum \epsilon_{h_1} \ldots \epsilon_{h_{s-1}} \epsilon_{l_1} \ldots \epsilon_{l_t}$ where summation is extended over the same indices $h_1, \ldots, h_s, l_1, \ldots, l_t$ as before. It follows that

$$S \leqslant 2^{-(p-1)} \eta (\sum \epsilon_{h_1}^2 \ldots \epsilon_{h_s-1}^2)^{1/2} (\sum \epsilon_{l_1}^2 \ldots \epsilon_{l_t}^2)^{1/2} \tag{10}$$

In $\sum \epsilon_{l_1}^2 \ldots \epsilon_{l_t}^2$ each term must be taken as many times as for given $l_1, l_2, \ldots, l_t$ one can find various groups of $h_1, h_2, \ldots, h_s$ satisfying (8). By (8) and Lemma 1, this sum is $\leqslant C^{s-1} \sum^* \epsilon_{l_1}^2 \ldots \epsilon_{l_t}^2$, the star meaning that now each term occurs once only. Since $\sum^* \epsilon_{l_1}^2 \ldots \epsilon_{l_t}^2 \leqslant (\epsilon_1^2 + \ldots + \epsilon_N^2)^t/t! = (2\lambda^2)^t/t!$, we get $\sum \epsilon_{l_1}^2 \ldots \epsilon_{l_t}^2 \leqslant 2^t C^{s-1} \lambda^{2t}/t!$ Similarly, in the sum $\sum \epsilon_{h_1}^2 \ldots \epsilon_{h_s-1}^2$ in (10) each term must be taken as many times as for given $h_1, h_2, \ldots, h_{s-1}$ one can find various groups of $l_1, \ldots, l_t$ satisfying (8). Since $h_s < l_t$, an application of Lemma 2 gives

$$\sum \epsilon_{h_1}^2 \ldots \epsilon_{h_s-1}^2 \leqslant C^t \sum^* \epsilon_{h_1}^2 \ldots \epsilon_{h_s-1}^2 = (\epsilon_1^2 + \ldots + \epsilon_N^2)^{s-1} C^t/(s-1)! = C^t (2\lambda^2)^{s-1}/(s-1)!$$

Hence

$$S \leqslant \eta (\lambda^2 C/2)^{(p-1)/2} / \{(s-1)!t!\}^{1/2} \leqslant \eta (\lambda^2 C/2)^{(p-1)/2} / \{[1/2(p-1)]!\}^{1/2}.$$

If we drop the last condition in (9), we have to double this estimate. If we vary $s$, $t$ but keep $p = s + t$ fixed, the result will be less than $p$ times the last expression. Finally, summing over $p$ and observing that the series with terms $(\lambda^2 C/2)^{(p-1)/2} p / \{[1/2(p-1)]!\}^{1/2}$ is uniformly convergent in $\lambda = O(1)$, we see that the total contribution of the terms so far considered is $\leqslant H\eta$, where $H$ is uniformly bounded for $\lambda = O(1)$. If we obtain a similar estimate for the terms so far disregarded, (iii) will be established.

But $s = 0$ implies $t \neq 0$ and can be omitted. If $t = 0$, we argue as before (by majorizing $\epsilon_{h_s}$ by $\eta$). The case $s = 1$ with $h_1 < l_t$ leads to $\gamma + n_{h_1} + \ldots + n_{l_t} - n_{h_1} = 0$ and is impossible; if $l_t < h_1$, the treatment is

as above. Finally, if $t = 1$ $(s > 1)$, and $n_{h_1} + \ldots + n_{h_s} = \gamma + n_{h_l}$, the case $h_s < l_l$ offers nothing new; if $h_s > l_1$, then $S \leqslant \eta 2^{-(p-1)} \sum \epsilon_{h_1} \ldots \epsilon_{h_s} \leqslant \eta 2^{-(p-1)} \{C^s \sum{}^* \epsilon_{h_1}{}^2 \ldots \epsilon_{h_s}{}^2\}^{1/2}$, and the argument concludes as before. Thus (iii) is proved.

Suppose now that (1) is of the class $L^2$. The remainder $(a_N \cos n_N x + b_N \sin n_N x) + \ldots$ of (1) represents then a certain function $R_N(x)$. By $G_N(y)$ we shall mean the distribution function of $R_N(x)/D_N$, where $D_N = \{1/2(a_N{}^2 + b_N{}^2) + \ldots\}^{1/2}$. The proofs of the following two results are repetitions of those of (i) and (iii).

(iv) *If* (1) *is of the class* $L^2$, *and if* $G_N(y)$ *tends to the distribution function* $G(y)$ *such that either* $G(y) > 0$ *or* $G(y) < 1$ *for all finite* $y$, *then* $c_N/D_N \to 0$.

(v) *Suppose that* (1) *is of the class* $L^2$, *that* $c_N/D_N \to 0$, *and let* $E$ *be a fixed set of positive measure. The distribution function of* $R_N(x)/D_N$ *relative to* $E$ *tends to the Gaussian distribution with mean value 0 and dispersion 1.*

Theorems (iii) and (v) have analogs for power series (2)

$$\sum_{k=1}^{\infty} c_k e^{in_k x} \qquad (n_{k+1}/n_k > q > 1) \tag{11}$$

We shall write $C_N = \{1/2(|c_1|^2 + \ldots + |c_N|^2)\}^{1/2}$, $D_N = \{1/2(|c_N|^2 + \ldots)\}^{1/2}$. The real and the imaginary part of the $N$th partial sum of (11) will be denoted by $U_N(x)$, $V_N(x)$, and of the $N$th remainder of (11) (if $C_N = O(1)$) by $U_N'(x)$, $V'_N(x)$, respectively.

(vi) *Let* $E$ *be a fixed set in* $(0, 2\pi)$ *of positive measure. If* $C_N \to \infty$, $c_N = o(C_N)$, *then the two dimensional distribution function, relative to* $E$, *of the pair of functions* $U_N(x)/C_N$, $V_N(x)/C_N$ *tends to* $(2\pi)^{-1} \int_{-\infty}^{\xi} \int_{-\infty}^{\eta} e^{-1/2(\lambda^2+\mu^2)} d\lambda d\mu$. *The same conclusion holds for* $U'_N(x)/D_N$, $V'_N(x)/D_N$ *if* (1) *is of the class* $L^2$ *and if* $c_N = o(D_N)$.

It is enough to sketch the proof of the first part of (vi). Let $Z_N(\xi, \eta)$ denote the set of points $x$ such that $U_N(x)/C_N \leqslant \xi$, $V_N(x)/C_N \leqslant \eta$, and let $F_N(\xi, \eta) = |Z_N(\xi, \eta)E|/|E|$. Let $c_k = |c_k| e^{i\alpha_k}$. The characteristic function of $F_N$ is

$$\int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} e^{i(\lambda\xi+\mu\eta)}\, dF_N(\xi, \eta) = |E|^{-1} \int_E e^{i(\lambda U_N + \mu V_N)/C_N}\, dx =$$
$$|E|^{-1} \int_E \exp.\, i\, C_N^{-1} \{\sum |c_k| (\lambda \cos(n_k x + \alpha_k) + \mu \sin(n_k x + \alpha_k)\} dx =$$
$$|E|^{-1} \int_E \exp. \{iC_N^{-1}(\lambda^2 + \mu^2)^{1/2} \sum |c_k| \cos(n_k x + \alpha'_k)\} dx$$

where the $\alpha_k'$ depend now on $\lambda$, $\mu$. As $N \to \infty$, and with an error $o(1)$, the last expression is

$$e^{-1/2(\lambda^2+\mu^2)} |E|^{-1} \int_E \prod_{k=1}^{N} \{1 + iC_N^{-1}(\lambda^2 + \mu^2)^{1/2} |c_k| \cos(n_k x + \alpha_k')\} dx$$
$$\to e^{-1/2(\lambda^2+\mu^2)}$$

which completes the proof.

The main idea used throughout this paper can be applied to other cases,

for example to linear combinations of the partial sums (or the remainders) of (1) and (11). We can also study the distribution functions in $(-\infty, +\infty)$ of the partial sums and of the remainders of the series $\sum c_k e^{i p_k t}$ with linearly independent exponents. We obtain the results parallel to the ones given above but with easier proofs.[3] The infinite products $\Pi(1 + i\alpha_k \cos n_k x)$ can also be used to obtain a simple proof of the theorem of Banach asserting the existence of continuous functions with prescribed Fourier coefficients at lacunary places. To these and other problems we shall return elsewhere.

[1] *Comptes Rendus Paris Acad. Sci.*, **224**, 516–878 (1947).
[2] See also *loc. cit.* (1).
[3] See also Kac and Steinhaus, *Studia Math.*, **7**, 1–15 (1938).

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## *TRANSFORMATION THEORY OF PHYSICAL CURVES*

BY EDWARD KASNER AND JOHN DE CICCO

DEPARTMENTS OF MATHEMATICS, COLUMBIA UNIVERSITY, NEW YORK, AND ILLINOIS INSTITUTE OF TECHNOLOGY, CHICAGO

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1. The importance of projective transformations[1] in dynamics was brought out by Appell in 1889. If $(\phi, \psi)$ are the rectangular components of any positional field of force in the plane, the corresponding equations of motion of a particle of unit mass are

$$\frac{d^2x}{dt^2} = \phi(x, y), \quad \frac{d^2y}{dt^2} = \psi(x, y). \tag{1}$$

If an arbitrary point transformation, unaccompanied by any change in the time, is applied, the new differential equations will usually involve not only $x$ and $y$, but also the velocity components $dx/dt$, $dy/dt$. In fact, the only exception is where the point transformation is merely affine.

Appell showed that if a general collineation

$$X = \frac{a_1x + b_1y + c_1}{a_0x + b_0y + c_0}, \quad Y = \frac{a_2x + b_2y + c_2}{a_0x + b_0y + c_0}, \tag{2}$$

is accompanied by a change of the time of the form

$$dT = \frac{dt}{k(a_0x + b_0y + c_0)^2} \tag{3}$$

the new differential equations will be of the original form

$$\frac{d^2X}{dT^2} = \Phi(X, Y), \quad \frac{d^2Y}{dT^2} = \Psi(X, Y), \tag{4}$$

and therefore define motion in some new positional field of force. The relation between the new field and the original field is explicitly as follows

$$\Phi = k^2(a_0x + b_0y + c_0)^2[C_2(y\phi - x\psi) + B_2\phi + A_2\psi],$$
$$\Psi = k^2(a_0x + b_0y + c_0)^2[C_1(y\phi - x\psi) + B_1\phi + A_1\psi], \tag{5}$$

where the capital letters denote minors in the determinant $(a_1b_2c_0)$.

The trajectories of the original field are converted by the collineation into the trajectories of the new field. Also the directions of the forces are projectively related. However, the vector transformation induced by the collineation between the two fields of force is *not* projective, but is actually a cubic transformation.

2. Appell proved the following converse theorem. The only transformations of the form

$$X = X(x, y), \quad Y = Y(x, y), \quad dT = F(x, y)dt, \tag{6}$$

which convert every set of differential equations of the form (1) into one of the same form are those defined by (2) and (3).

3. Upon eliminating the time $t$ from the differential equations (1), the differential equation of third order defining the $\infty^3$ trajectories of a positional field of force is

$$(\psi - y'\phi)y''' = [\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2]y'' - 3\phi y''^2. \tag{7}$$

Kasner proved that *the only point transformations (or contact transformations) which convert every trajectory system of a positional field of force defined by a third order differential equation of the form* (7) *into a trajectory system of the same form are the collineations.*[2]

4. We shall consider the transformation theory of other noteworthy systems of curves connected with a positional field of force, namely, brachistochrones, catenaries and velocity curves. In order to do this in a simple manner, it is found convenient to discuss the transformation theory of certain systems $S_k$ which include the four systems of physical interest as special cases.[3]

A *system* $S_k$ in a given positional field of force consists of curves along which a constrained motion is possible so that the pressure $P$ is proportional to the normal component $N$ of the force vector. Thus $P = kN$. The differential equation of third order defining, a system $S_k$ of $\infty^3$ curves is

$$(\psi - y'\phi)y''' = [\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2]y'' - \left[3\phi + \frac{(n-2)(\phi + y'\psi)}{1 + y'^2}\right]y''^2, \tag{8}$$

where

$$n = \frac{2}{k+1}. \tag{9}$$

The *four cases of physical interest are*

$k = 0$ or $n = 2$ gives $S_0$, the system of *trajectories*.
$k = -2$ or $n = -2$ gives $S_{-2}$, the system of *brachistochrones*.
$k = 1$ or $n = 1$ gives $S_1$, the system of *catenaries*.
$k = \infty$ or $n = 0$ gives $S_\infty$, the system of *velocity curves*.

5. We shall establish the following result.

*Except for the system $S_0$ of trajectories, the only point transformations (or contact transformations) which convert every system $S_k$ of curves into a system $S_k$ are the similitudes.*

In particular, the similitudes are the only contact transformations which carry every system of brachistochrones, or catenaries, or velocity curves into a system of brachistochrones, or catenaries, or velocity systems, respectively.

6. In order to prove this result, we observe in the first place that any system $S_k$ possesses the Property I (that is, the focal locus of the osculating parabolas of the trajectories passing through a given lineal element $E$ is a circle passing through the point of $E$). Any system of curves with the Property I is said to be of the *type* $(G)$. This $(G)$ type is defined by the differential equation of third order

$$y''' = G(x, y, y')y'' + H(x, y, y')y''^2. \tag{10}$$

Kasner proved that *the only contact transformations which send every system of $\infty^3$ curves of the type $(G)$ into a system of the type $(G)$ are the collineations and correlations.*

7. We shall verify that the collineation (2) actually leaves the type $(G)$ unchanged.[4]

The first three extensions of the collineation (2) are

$$Y' = \frac{dY}{dX} = \frac{C_1(y - xy') + B_1 + A_1y'}{C_2(y - xy') + B_2 + A_2y'}$$

$$Y'' = \frac{d^2Y}{dX^2} = (a_1b_2c_0)\Omega^3y'', \quad \Omega = \frac{a_0x + b_0y + c_0}{c_2(y - xy') + B_2 + A_2y'}$$

$$Y''' = \frac{d^3Y}{dX^3} = (a_1b_2c_0)(a_0x + b_0y + c_0)\Omega^4y''' + 3(a_1b_2c_0)(xC_2 - A_2)\Omega^5y''^2 + 3(a_1b_2c_0)(a_0 + b_0y')\Omega^4y''. \tag{11}$$

Let the $(G)$ type in the $(X, Y)$-plane be

$$Y''' = \bar{G}(X, Y, Y')Y'' + \bar{H}(X, Y, Y')Y''^2 \qquad (12)$$

By (11), it is found that under a collineation this corresponds to a $(G)$ type (10) in the $(x, y)$-plane. The correspondence between the defining functions is

$$G = \frac{1}{a_0x + b_0y + c_0}\left[\frac{\bar{G}}{\Omega} - 3(a_0 + b_0y')\right],$$

$$H = \frac{\Omega}{a_0x + b_0y + c_0}\left[(a_1b_2c_0)\Omega\bar{H} - 3(xC_2 - A_2)\right]. \qquad (13)$$

8. The calculation for correlations is simplified by observing that every correlation may be reduced by means of collineations to Legendre's transformation

$$X = -y', \quad Y = xy' - y, \quad Y' = -x. \qquad (14)$$

This is polarity with respect to the conic $x^2 + 2y = 0$. Extending this, we find

$$Y'' = \frac{1}{y''}, \quad Y''' = \frac{y'''}{y''^3}. \qquad (15)$$

This converts the $(G)$ type (10) into the $(G)$ type (12) where the new coefficient functions are related to the old as follows

$$G = \bar{H}(-y, xy' - y, x), \quad H = \bar{G}(-y, xy' - y, -x). \qquad (16)$$

Thus we have verified that the collineations and correlations leave the $(G)$ type unchanged. Therefore the only possible contact transformations that send every system $S_k$ of $\infty^3$ curves into a system $S_k$ are the collineations and correlations.

9. Next we observe that any system $S_k$ possesses the Property II, which may be stated in the following manner. At any point $O$, the tangent of the angle which the focal circle makes with the given element is to the tangent of the angle which the given element makes with a certain direction fixed at $O$ of slope $\omega(x, y)$ (the direction of the acting force), as 3 is to $n + 1$, that is, as $3k + 3$ is to $k + 3$. This means analytically that $H$ has the form

$$H = \frac{3}{y' - \omega} + \frac{(n - 2)(1 + y'\omega)}{(1 + y'^2)(y' - \omega)}. \qquad (17)$$

*It is evident that the correlations do not preserve systems with Properties I and II.* For $H$ and $G$ are interchanged under the Legendre transformation and hence the special function $H$ becomes an arbitrary function $G$.

*Finally the only collineations which preserve the systems with Properties*

*I and II where $k \neq 0$ or $n \neq 2$ are the similitudes.* For by (17), $Y' = \pm i$ renders $\overline{H}$ infinite. Then by the last of equations (13), the pre-images of the minimal lines $Y' = \pm i$ must make $H$ infinite. But we know by (17) that $y' = \pm i$ are infinite roots of $H$. Thus the minimal lines $y' = \pm i$ must be converted into the minimal lines $Y' = \pm i$ by the collineation (2) (unless the field of force has constant slope $\pm i$ which possibility is excluded from consideration). Therefore any such collineation is necessarily a similitude.

As it is evident that similitudes do preserve the systems $S_k$, it follows that the proof of the Theorem of Section 5 is complete.

[1] Appell, *Amer. Jour. Math.*, 1889. The importance of the conformal group in dynamics (conservative fields of force) has been emphasized by Larmor, Goursat and Darboux.

[2] Kasner, *Differential-Geometric Aspects of Dynamics*, Princeton Colloquium Lectures, American Mathematical Society Publications 1913, 1934.

[3] Kasner, "Physical Curves," these PROCEEDINGS, **33**, 346–351 (1947).

[4] Kasner, "The Trajectories of Dynamics," *Trans. Amer. Math. Soc.*, **7**, 401–424 (1906).

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## *THE ENERGY SOURCE FOR BIOLUMINESCENCE IN AN ISOLATED SYSTEM*

BY WILLIAM D. McELROY*

DEPARTMENT OF BIOLOGY, JOHNS HOPKINS UNIVERSITY

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When luminous organisms are extracted with water or saline solutions one usually obtains in the crude preparation a temporary emission of light which disappears rapidly. In all but five of some twenty-one groups of luminous forms the light-emitting system is destroyed by the extraction procedures. Some of the difficulties are exemplified by the system which has been extracted from the crustacean, *Cypridina hilgendorfii*. Here, Harvey[1] demonstrated that the substrate for the reaction, luciferin, was highly unstable in the presence of air. The oxidation which occurred, however, could be reversed by simple reductants provided the compound did not remain in the oxidized state for too long a period. In the presence of the enzyme, luciferase, however, an oxidation occurred, accompanied by light production, which was not reversible by simple reductants.[2] The degradation of a ketohydroxy side chain on the luciferin molecule has been presented as a possible explanation of this irreversible reaction.[3] Since purified *Cypridina* luciferin was shown to contain labile phosphate, it has been postulated that the energy derived from the breakdown of the side chain is conserved as phosphate bond energy.[4] This suggestion accounts

satisfactorily not only for the irreversible reaction observed *in vitro* but also for the energy requirements of luminescence. The hypothesis was supported by the observation that phosphate was released during the luminescent reaction.

Recently results have been obtained with Lampyrid beetles, popularly known as "fireflies," which support the early suggestion that labile phosphate is concerned in the luminescent reaction. When live fireflies (*Photinus pyralis*) are ground with sand and water in a mortar one obtains momentarily a preparation which is highly luminous; however, the light disappears rapidly with continued grinding. Presumably the non-luminous extract contains the enzyme luciferase, and the luciferin which has been irreversibly decomposed. However, if one adds a small amount of adenosine triphosphate (ATP)[5] to this crude extract a brilliant flash of light appears immediately and lasts for a considerable time depending on the ATP concentration. The results summarized in figure 1 showing the

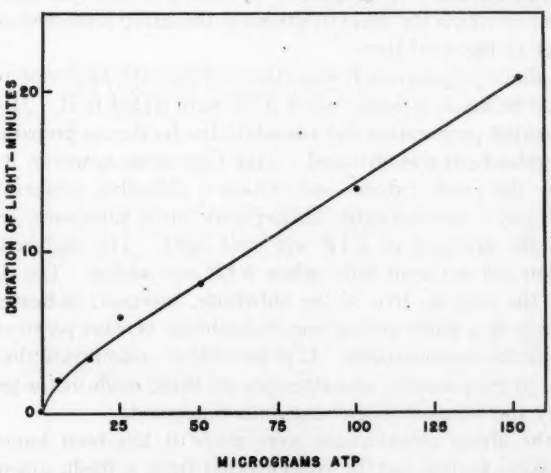


FIGURE 1

The relationship between light emission and adenosine triphosphate

relationship between ATP concentration and duration of light were obtained with the following preparations. The fireflies were placed for 30 minutes in absolute ethyl alcohol containing dry ice and then dried under vacuum in the frozen state. After drying, the lanterns were removed from 50 fireflies and ground with sand and 15 ml. of cold water for 10 minutes. The preparation was centrifuged for 20 minutes (R.C.F. = 2000) and the supernatant, which presumably contained luciferin, was saved. To

obtain the enzyme 50 live fireflies were ground with sand and 50 ml. of water and finally centrifuged as above and the supernatant saved. When the two extracts were combined no light appeared. However, with the addition of ATP the entire preparation gave what appeared to be a very homogeneous glow. In the above experiments 0.1 ml. of the substrate was mixed with 0.1 ml. of enzyme solution. Water or ATP was added to give a final volume of 0.7 ml.

At the present time it is not possible to measure the total amount of light emitted by a given concentration of ATP. Therefore, one cannot make a quantitative comparison between light and ATP. With low concentrations of ATP a very bright light is first observed which then decays slowly and finally disappears. With higher concentrations of ATP a bright light is maintained fairly constant for a while and then decays slowly. Consequently, with low concentrations of ATP which maintain the light for only a few minutes a large percentage of the observation time is concerned with the decay part of the curve in contrast to higher concentrations in which the decay portions of the curve represent only a small percentage of the total time.

In the above preparation it was observed that the luciferase preparation itself could be made to luminesce if ATP were added to it. However, the alcohol-treated preparation did stimulate the luciferase preparation and a much brighter light was obtained. Like *Cypridina*, however, it is possible to dialyze the crude extract and obtain a diffusible, temperature-stable substrate and a non-diffusible temperature-labile substance, which when mixed in the presence of ATP will emit light. The dialyzed luciferase preparation did not emit light when ATP was added. The difficulty of dialyzing the enzyme free of the substrate, however, indicates that the combination is a fairly strong one, resembling certain prosthetic groups and apoenzyme combinations. It is possible to concentrate the dialyzable substance by evaporation, and attempts are being made at the present time to identify the compound or compounds concerned.

Since the above observations were made it has been found that the light emitting system can be concentrated from a fresh aqueous extract of the fireflies simply by drying the extract under vacuum. Likewise, whole flies can be dried under vacuum and an extract made of the dried lanterns. However, if the whole fireflies are kept in the dried state for several days the extracted system cannot be made to luminesce with the addition of ATP even though a bright light is obtained during the extraction procedure. This is in contrast to the dried extract made from fresh material which always emits light with the addition of ATP, even after remaining in the dried state for over a month. It has also been observed that oxygen is necessary for light emission in the firefly extract, for when the various components are mixed under anaerobic conditions no light is

obtained. With the admission of air, however, the usual bright glow appears immediately.

It is not possible at the present time to say whether ATP is concerned directly or indirectly with light production. With the crude preparations it is conceivable that ATP is a necessary component for some system which in turn "re-activates" the luciferin. However, the earlier observations that acid-labile phosphate is present in the purified *Cypridina* luciferin preparations and that phosphate is released concomitantly with light emission lead one to suspect that the "energy rich" phosphate groups are directly concerned in exciting the luciferin molecule. On the basis of the previous hypothesis, then, it is suggested that the light-emitting reaction is reversed by the transfer of phosphate from ATP to the luciferin molecule, the energy requirements for light emission being satisfied primarily by phosphate bond energy. This suggests that luciferin may act in both phosphate and electron transporting systems.[6]

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[1] The numerous studies on bioluminescence are discussed by E. Newton Harvey, *Living Light*, Princeton University Press, Princeton, N. J., 1940.

[2] Anderson, R. S., *J. Gen. Physiol.*, **19**, 301–305 (1935).

[3] Chakravorty, P. N., and Ballentine, R., *J. Am. Chem. Soc.*, **63**, 2030 (1941).

[4] McElroy, W. D., and Ballentine, Robert, *Proc. Nat. Acad. Science*, **30**, 377–382 (1944).

[5] The adenosine triphosphate was prepared from rabbit muscle as the barium salt according to the method of Needham, using the modifications given in Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *Manometric Techniques*, Burgess Publishing Co., Minneapolis, 1945. According to the 7 minute phosphate and purine analysis the preparation was 85% pure.

[6] I wish to thank Drs. E. N. Harvey, A. M. Chase, R. S. Anderson and Robert Ballentine for suggestions made in the preparation of the manuscript and Dr. John B. Buck for identifying the species of fireflies.